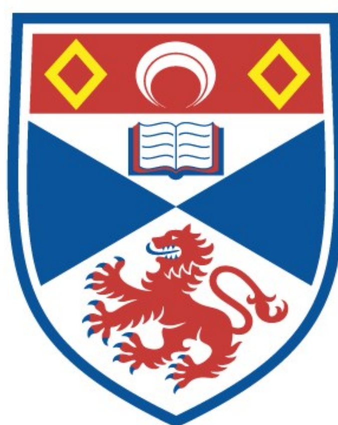
SYNTHESIS OF POTENTIAL SUBSTRATES AND INHIBITORS FOR PYRIDOXAL-PHOSPHATE DEPENDENT ENZYMES

Khurshida Khayer

A Thesis Submitted for the Degree of MPhil
at the
University of St Andrews



1997

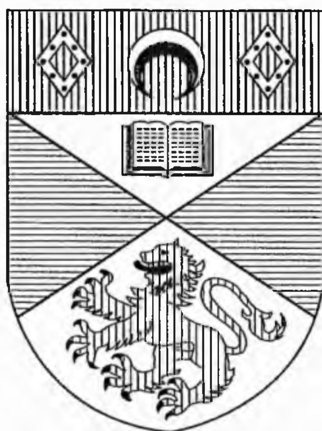
Full metadata for this item is available in
St Andrews Research Repository
at:
http://research-repository.st-andrews.ac.uk/

Please use this identifier to cite or link to this item:
http://hdl.handle.net/10023/14385

This item is protected by original copyright

SYNTHESIS OF POTENTIAL SUBSTRATES AND INHIBITORS FOR PYRIDOXAL-PHOSPHATE DEPENDENT ENZYMES

KHURSHIDA KHAYER



Thesis submitted to the University of St. Andrews for the degree of Master of Philosophy

ProQuest Number: 10166367

All rights reserved

INFORMATION TO ALL USERS
The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript
and there are missing pages, these will be noted. Also, if material had to be removed,
a note will indicate the deletion.

ProQuest 10166367

Published by ProQuest LLC (2017). Copyright of the Dissertation is held by the Author.

All rights reserved.
This work is protected against unauthorized copying under Title 17, United States Code
Microform Edition © ProQuest LLC.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

Dedicated To

My Husband and the Memory of My Father with Eternal Love

Abstract

Pyridoxal 5'-phosphate dependent aspartate aminotransferase catalyses the conversion of (2*S*)-aspartic acid and α-ketoglutaric acid into (2*S*)-glutamic acid and oxaloacetate. Both the mono and dimethylesters of (2*S*)-aspartic acid, together with the corresponding amides were synthesised in order to assess the influence of charge neutralisation of the α-amino pK of aspartate bound to aspartate aminotransferase. A novel method was developed for the synthesis of the (2*S*)-aspartic acid α-methylester (**57**) starting from the β-allyl ester of aspartic acid. The required ester (**57**) was obtained in 25% yield over 4 steps. pKa Studies carried out in collaboration with Profs. Schnackerz and Cook, have revealed that, although both of the carboxylates of aspartate form hydrogen-bonds to Arg-292 and Arg-386, upon binding to the enzyme, charge is not completely neutralised at either of the two.

Glutamate 1-semialdehyde aminomutase is an exceptional PLP-dependent enzyme in that, although it is classified as a mutase (transfering amino and oxo functions within the molecule) it functions as an aminotransferase. We have devised a new route to 2,3-diaminopropyl hydrogen sulfate, a substrate/ inhibitor which should shed more light on the mechanism of this enzyme. The key steps in the synthesis were the conversion of the amide (**68**) into the amine (**69**) using the Waki modification of the Hoffman reaction and reduction of the α-carboxylate into the primary alcohol (**53**). Sulfation of the diamino alcohol (**66**) using chlorosulfonic acid in DCM gave the required sulfate (**56**), contaminated with upto 20% of starting material. The material was purified by ion exchange chromatography and was used in kinetic studies in the laboratory of Prof. R. John.

We have also attempted to develop a new synthetic route to 3-[^{2}H]-(2*S*)-serine starting from (2*S*)-aspartic acid. This compound should be useful in delineating the mechanism of a number of other PLP-dependent enzymes. Although we have synthesised a number of Baeyer-Villiger precursors we have been unable to find conditions in which the oxidation proceeds in more than 20% yield.

DECLARATIONS

I, Khurshida Khayer, hereby certify that this thesis, which is approximately 20,000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

Date:...18.9.1996.......... Signature of candidate:...............................

POSTGRADUATE CAREER

I was admitted as a research student in July 1995 and as a candidate for the degree of M. Phil. in July 1995; the higher study for which this is a record was carried out in the university of St. Andrews between 1995 and 1996.

Date:...18.9.1996...... Signature of candidate:..............................

CERTIFICATE

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of M.Phil. in the University of St. Andrews and that the candidate is qualified to submit this thesis in application for that degree.

Date: 18 September 1996 Signature of supervisor: ....

COPYRIGHT

In submitting this thesis to the University of St. Andrews I understand that I am giving permission for it to be made available for the use in accordance with the regulations of the University Library for the time being in force, subject to any copyright vested in the work not being affected thereby. I also understand that the title and the abstract will be published, and that a copy of the work may be made and supplied to any *bona fide* library or research worker.

Date:....18.9.1996..... Signature of candidate:.............................

Acknowledgements

It is a matter of great pleasure for me to record my deepest sense of gratitude, honour and thanks to my supervisors, Professor David Gani and Dr. Mahmoud Akhtar for their constant guidance, valuable suggestions and encouragement throughout the course of this work.

My time in St. Andrews has been fulfilling, having made many friends and colleagues. I desire to express my gratitude and thank to my friend Morag (Dr. M. Lenman), Thierry (Dr. T. Jenn), Jurgen (Dr. J. Schulz) and Tony (Dr. T. Maude) for their kind co-operation and friendly behaviour during the course of this work. My special thanks and appreciations must go to Arwel, Roger, Nigel and Martin for making such a friendly environment in the Lab 434.

I would also like to give thanks to Julie, Darren, Amit, Mark, Stacey, Donald, Kerri and Panthea for helping me 'come to terms' with the Apple Macintosh computer during typing of this manuscript. Thanks also go to all members of the School of Chemistry for their help, advice and technical assistance specially to Colin and Caroline.

I am very grateful to Prof. Mesbahuddin Ahmed and Prof. Rabiul Islam who inspired me to study here.

I gratefully acknowledge the School of Chemistry at the University of St. Andrews for financial support and Jahangirnagar University for giving me the extra-ordinary study leave during the course of this work.

Finally, I recognise the contributions of all my family members for their love, inspiration and sacrifice during the course of this work.

Contents

Abstract i
Acknowledgements vi

Chapter 1

1.0 Introduction 1
1.1 Metabolic role of PLP Dependent Enzymes 2
1.2 Structure and Mechanism of PLP Dependent Enzymes 7
1.3 Reactions at the α-Carbons 9
1.3.1 Transamination 9
1.3.2 Glutamate 1-Semialdehyde Aminomutase 16
1.3.3 Racemization 21
1.3.4 Decarboxylation 22
1.3.5 C^{α}-R Bond Cleavage or Formation 23
1.4 Reactions at the β- and γ-Carbon 24
1.5 Suicide Inhibitors for PLP Dependent Enzymes 26
1.5.1 Transaminase Inhibitors 27
1.5.2 Other Inhibitors 31

Chapter 2

2.0 Results and Discussion 37
2.1 Synthesis of α- and /or β-methyl aspartate ester and its analouges substituted at α- and /β-carboxylate 37
2.2 Synthesis of 2,3-diaminopropyl hydrogensulfate 47
2.2.1 Future work 52
2.3 Synthesis of 3-[^{2}H]-Serine 53
2.3.1 Future work 60

Chapter 3

3.0 Experimental 61

Chapter 4

4.0 References 84

Abbreviation	Meaning
AAT	Aspartate aminotransferase
ALA	5-Aminolevulinate
CBZ	Carbobenzyloxycarbonyl
CNS	Central Nervous System
DAVA	4,5-Diaminovalerate
DOPA	Dihydroxyphenylalanine
DFMO	α,α-Difluoromethylornithine
DMF	*N,N*-Dimethylformamide
DMSO	Dimethylsulfoxide
DOPA	3,4-Dihydroxyphenylalanine
EC	Enzyme Catalogue
E. coli	Escherichia coli
E_L	Enzyme with coenzyme as an internal aldimine of pyridoxal 5'-phosphate
E_M	Enzyme with coenzyme as pyridoxamine 5'-phosphate
GABA	4-Aminobutanoic acid (γ-aminobutyric acid)
GABA-T	γ-Aminobutyric acid transaminase
GSA	Glutamate 1-semialdehyde
GSA-AT	Glutamate 1-semialdehyde aminotransferase
GSA-AM	Glutamate 1-semialdehyde aminomutase
HDC	Histidine decarboxylase
k_{cat}	Enzymic catalytic constant/turnover number
K_i	Enzymic inhibition constant
M_r	Relative molecular mass
NMR	Nuclear magnetic resonance
ODC	Ornithine decarboxylase
PLP	Pyridoxal 5'-phosphate

PMP	Pyridoxamine 5'-phosphate
SAM	S-Adenosylmethionine
SHMT	Serine hydroxymethyltransferase
THF	Tetrahydrofuran
TLC	Thin layer chromatography
UV	Ultra violet

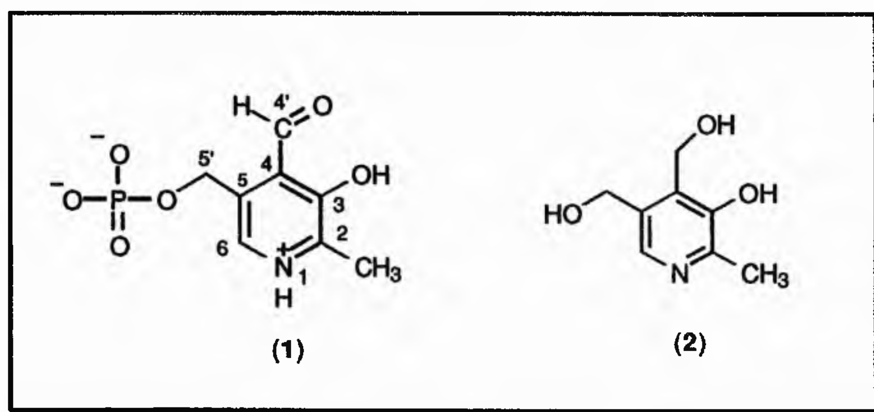
Amino acid	Three letter code abbreviation	Single letter code abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Chapter One

INTRODUCTION

1.0. Introduction

Pyridoxal 5'-phosphate (PLP, **1**), the biologically active form of vitamin B_6 (Pyridoxol, **2**) is one of the nature's most versatile co-enzymes and serves as a cofactor for a myriad of enzyme-catalyzed reactions, many of which are essential for amino acid metabolism. The enzymes have several common mechanistic and stereochemical features. In the mid 1940's Snell[1] identified PLP as the coenzyme for transaminases, and since then, PLP has been established as the coenzyme for over 20 different types of enzyme catalysed reactions.



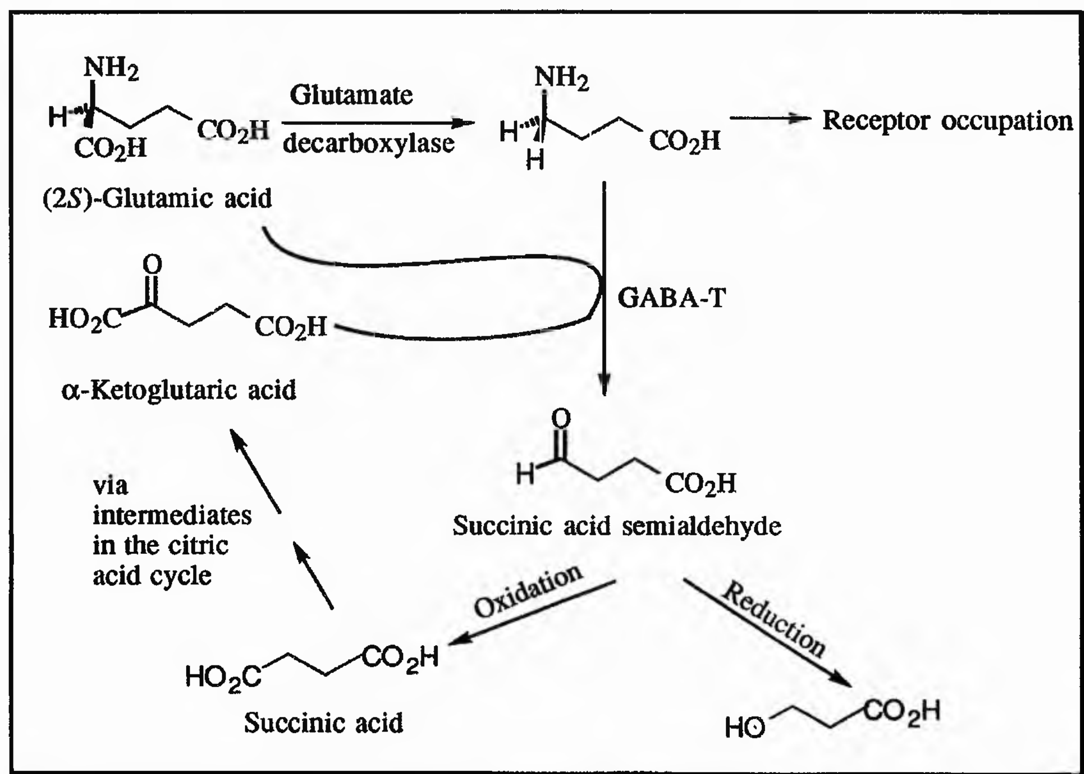
PLP is the required cofactor for enzymes integral in connecting the carbon and nitrogen cycles. The coenzyme is also involved in the formation of biogenic amines and in providing entry into the "one-carbon pool". The fact that PLP dependent enzymes occur in four of the six EC classes reflects its versatility.[2]

Experimental B_6 deficiency in animals results in vitamin B_6 pellagra, characterized by loss of hair, edema and red, scaly skin. Deficiency inhibits the degradation of (2*S*)-tryptophan, and the excretion of xanthurenic acid, a product of PLP-dependent tryptophan catabolism) is used as an index of vitamin B_6 deficiency.

1.1 Metabolic role of PLP dependent enzymes

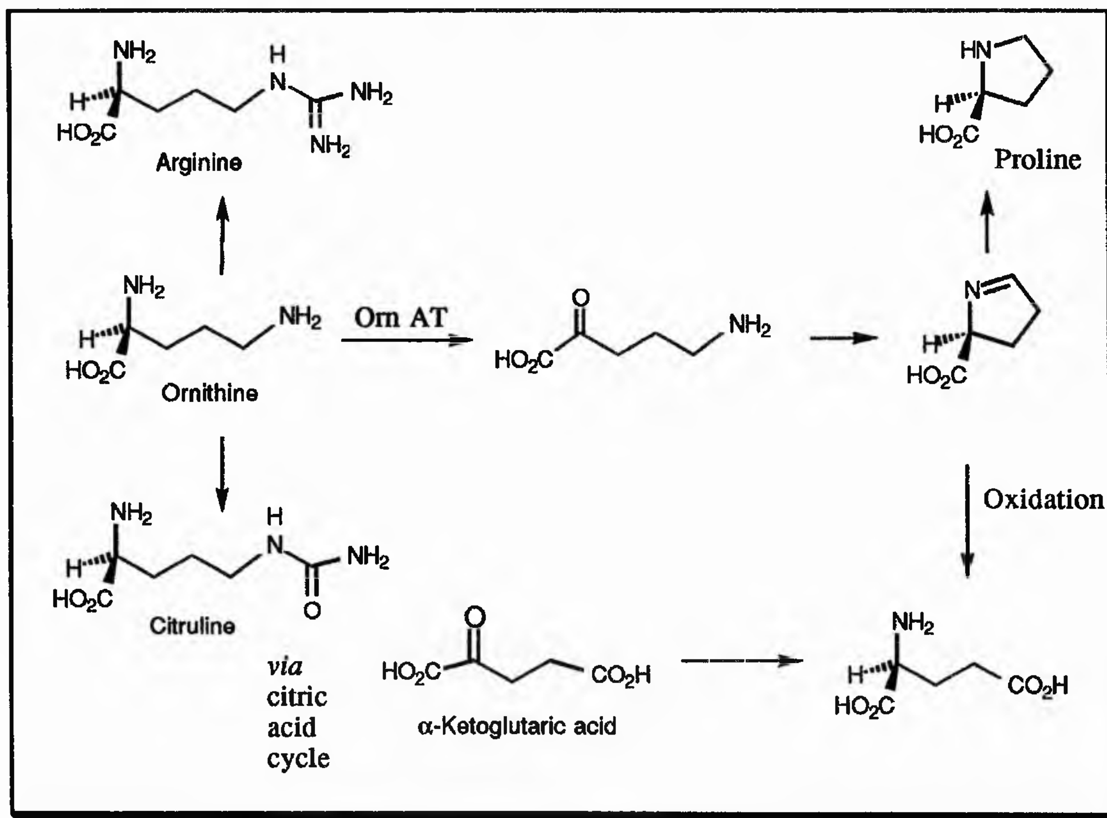
There has been a great deal of interest in understanding the mechanism of action of PLP dependent enzymes because of the important role they play in many physiological processes. Some illustrative examples are discussed below.

γ-Aminobutyric transaminase (GABA-T) is involved in the regulation of the GABA-ergic system[3] in the mammalian central nervous system (CNS) (Scheme 1.1). γ-Aminobutyric acid (GABA) is a inhibitory neurotransmitter and there is much evidence to suggest that high cerebral concentrations of this compound prevent convulsions, by depressing the firing of neurons throughout the CNS.



Scheme 1.1: *The GABA-ergic system.*

ω-Ornithine transaminase not only interconnects the citric acid cycle and urea cycle but also couples ornithine to proline metabolism (Scheme 1.2).[4]



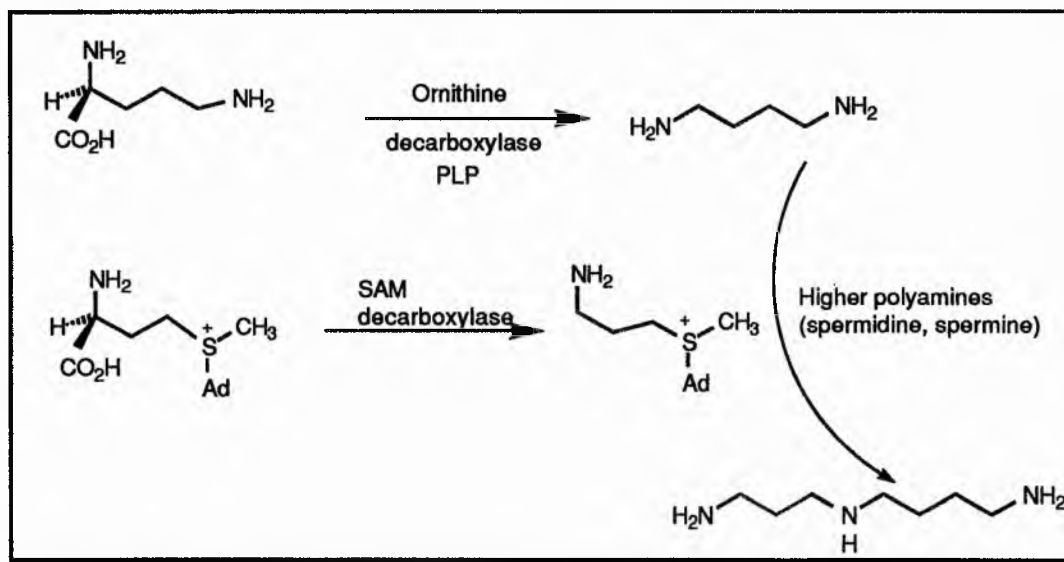
Scheme 1.2: *The metabolic significance of ornithine transaminase.*

The aminomutase, glutamate 1-semialdehyde aminomutase (GSA-AM), also called glutamate 1-semialdehyde aminotransferase (GSA-AT) catalyses the formation of δ-aminolevulinate (see Schemes 1.8 and 1.9; p.16-20), and it is the last enzyme in the t-RNA^{Glu}-mediated pathway that produces δ-aminolevulinate for the synthesis of chlorophyll, heme, and other tetrapyrrole pigments.[5]

One of the most important physiological roles of the decarboxylases in mammals is the conversion of amino acids to pharmacologically active amines.[6] The formation of dopamine, histamine, GABA, and putrescine all depend on PLP-dependent decarboxylation of their respective parent amino acids. The biosynthesis of higher

polyamines, spermine and spermidine, from putrescine involve the pyruvate-dependent decarboxylation of S-adenosyl methionine (SAM). Mammalian brain glutamate decarboxylase is directly responsible for the biosynthesis of GABA in the GABA-ergic system (Scheme 1.1).[3]

Ornithine decarboxylase (ODC) is the first and rate limiting enzyme in the biosynthetic pathway leading to putrescine and higher polyamines (Scheme 1.3).[4] The activity of the enzyme *in vivo* increases dramatically in response to cellular stimulation which promotes regeneration and replication. Cellular levels of the enzyme are high during growth, and low at other stages.[7] The association of high polyamine levels with rapid cellular proliferation and protein biosynthesis, led to the idea that polyamines may be required for RNA/DNA biosynthesis.[8] The enzyme has been identified as a target for cancer chemotherapy.[4]



Scheme 1.3: *The synthesis of higher polyamines.*

Histidine decarboxylase (HDC) catalyses the decarboxylation of histidine to give histamine. In mammals, histidine is important as the major receptor agonist.[9] Overproduction of histamine is associated with many biological processes including gastric secretion, allergic and hypersensitivity reactions.[10] The development of

antihistamines (antagonists for the receptors), has been an important area in medicinal chemistry. Histamine is also active as a regulator of peripheral blood circulation.[10] The aromatic amino acid decarboxylases catalyse the formation of many pharmacologically important amines. For example, DOPA decarboxylase is able to convert many aromatic substrates to the corresponding amines including, phenylalanine (**3**) to phenylethylamine (**4**), tyrosine (**5**) to tyramine (**6**), tryptophan (**7**) to tryptamine (**8**), histidine (**9**) to histamine (**10**), 3,4-dihydroxyphenylalanine (DOPA) (**11**) to 3,4-dihydroxyphenylethylamine (dopamine) (**12**) and 5-hydroxytrytophan (**13**) to serotonin (**14**), Table 1.1.[11] Dopamine and serotonin act on smooth muscles and the cardiovascular system. Dopamine is a precursor in the biosynthesis of adrenaline and serotonin itself is neuroactive.[10]

Another important class of PLP dependent enzymes, the racemases, catalyses the interconversion of (2*R*)- and (2*S*)- amino acids. These enzymes are common in the prokaryotes and several are involved in the biosynthesis of peptidoglycan in bacterial cell walls.[12] Alanine racemase is an important enzyme in peptidoglycan biosynthesis and therefore is a target for the action of many antibacterial agents,[13] including (2*R*)-cycloserine (**15**)[15], O-carbamyl-(2*R*)-serine (**16**) and phosphoalanine (**17**).[13]

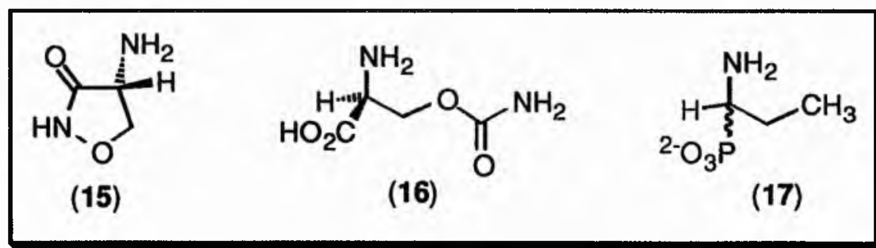
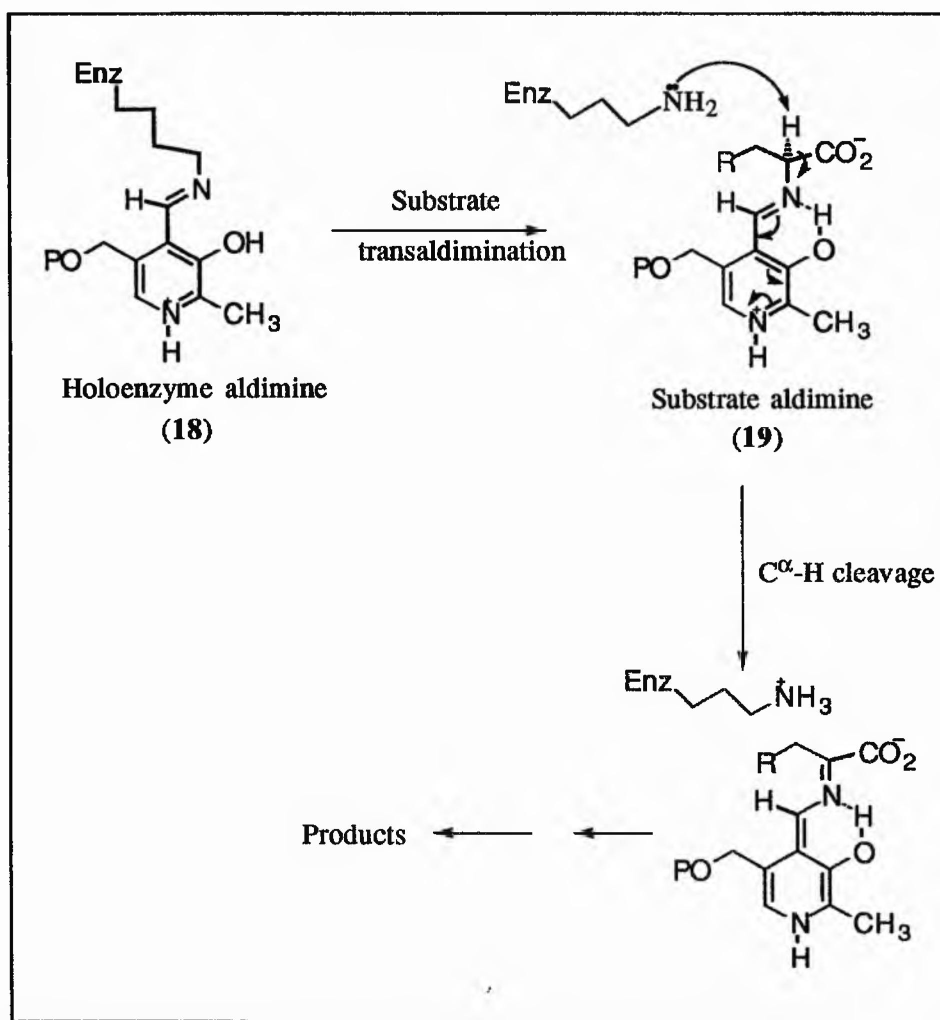


Table 1.1: *Some of the aromatic substrates decarboxylated by DOPA decarboxylase*

Substrate	Product amine
(3)	(4)
(5)	(6)
(7)	(8)
(9)	(10)
(11)	(12)
(13)	(14)

1.2 Structure and mechanism of PLP dependent enzymes

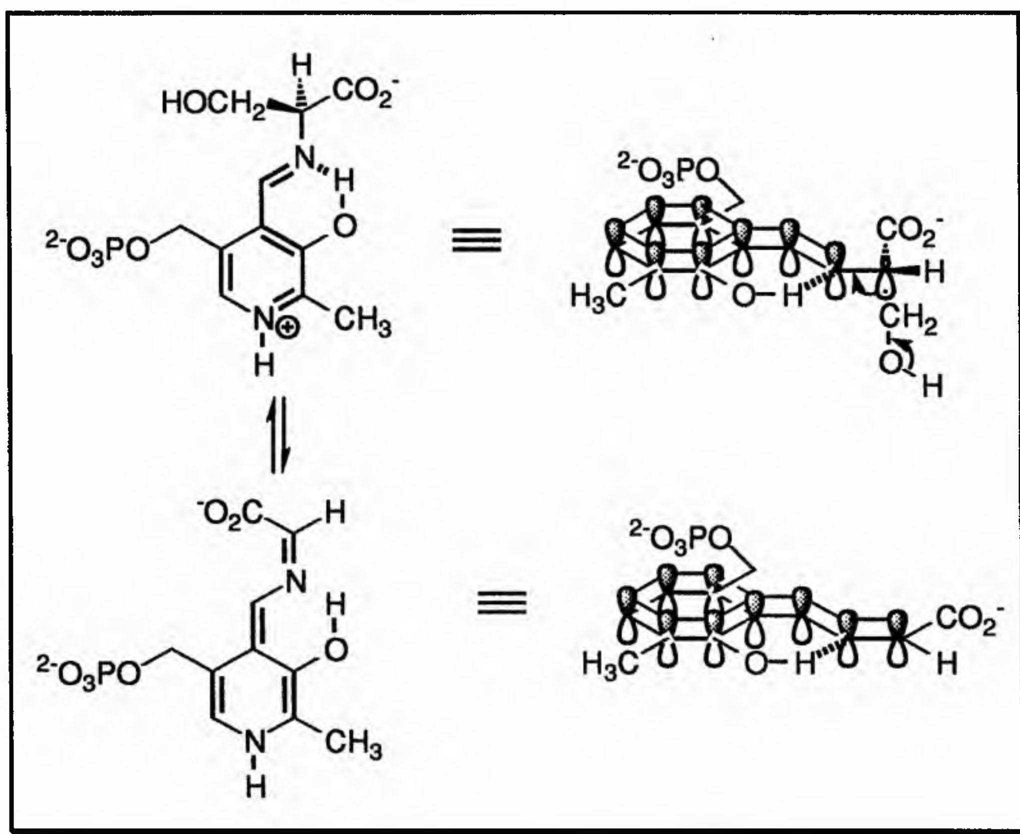
The general theory of the PLP mediated amino acid reactions was developed independently by Braunstein and Snell in 1953-54.[15,16] The co-enzyme PLP, is bound at the active site as a Schiff's base complex (**18**) with the ε-amino group of an active site lysine residue (Scheme 1.4).[17-19] Introduction of the substrate leads to a transaldimination reaction which frees the active site lysine residue and leads to the formation of another Schiff's base, substrate aldimine (**19**) (Scheme 1.4). For some enzymic systems the transaldimination reaction causes a large conformational change in the conformation of the co-enzyme complex at the active site of the enzyme.



Scheme 1.4: *PLP and the conjugated electron-deficient π-electron system shown for the removal of C^{α}-H from a (2S)-amino acid.*

In the presence of the appropriate enzyme, all of the bonds connected to the C^{α} of the imino acid moiety can be cleaved.

Dunathan[20] proposed that after formation of the enzyme-substrate Schiff's base complex (**19**) the torsional angle about the N-C^{α} bond is fixed by the enzyme so that the bond to be cleaved at C^{α} is held at 90° to the plane of the pyridinium ring. In this conformation, maximum orbital overlap is achieved between the developing charge and the conjugated electron-deficient π-electron system (Scheme 1.4). The cleavage is further facilitated by an enzyme-bound base, usually the ε-amino group of the lysyl residue, which stabilize the positive charge as it forms on the electrofuge, in this case a proton. Thus the PLP dependent enzymic reactions vary depending on which of the bonds at C^{α} is to be cleaved (Scheme 1.5).



Scheme 1.5: *Preferential cleavage of the bond orthogonal to the plane of the pyridinium ring, as predicted by the Dunathan hypothesis. This example shows C^{α}-CH_2OH cleavage, but C^{α}-CO_2^- and C^{α}-H bond cleavage (i.e. decarboxylation and transamination/racemisation reactions respectively) can also occur.*

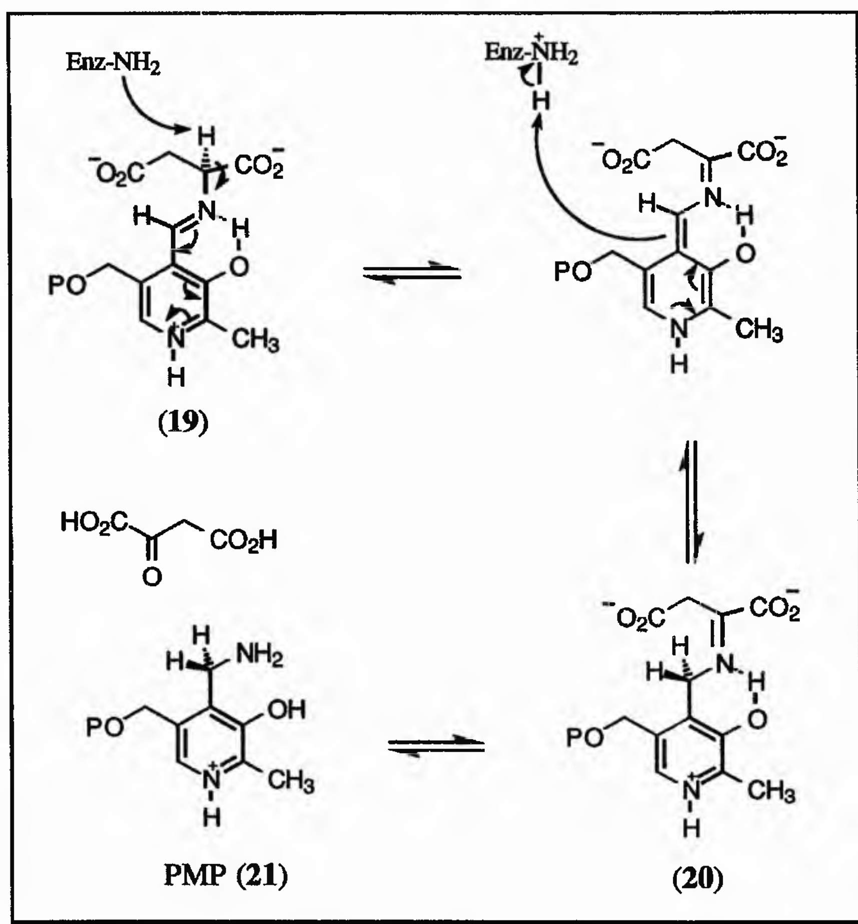
1.3 Reactions at the α-Carbons

1.3.1 Transamination

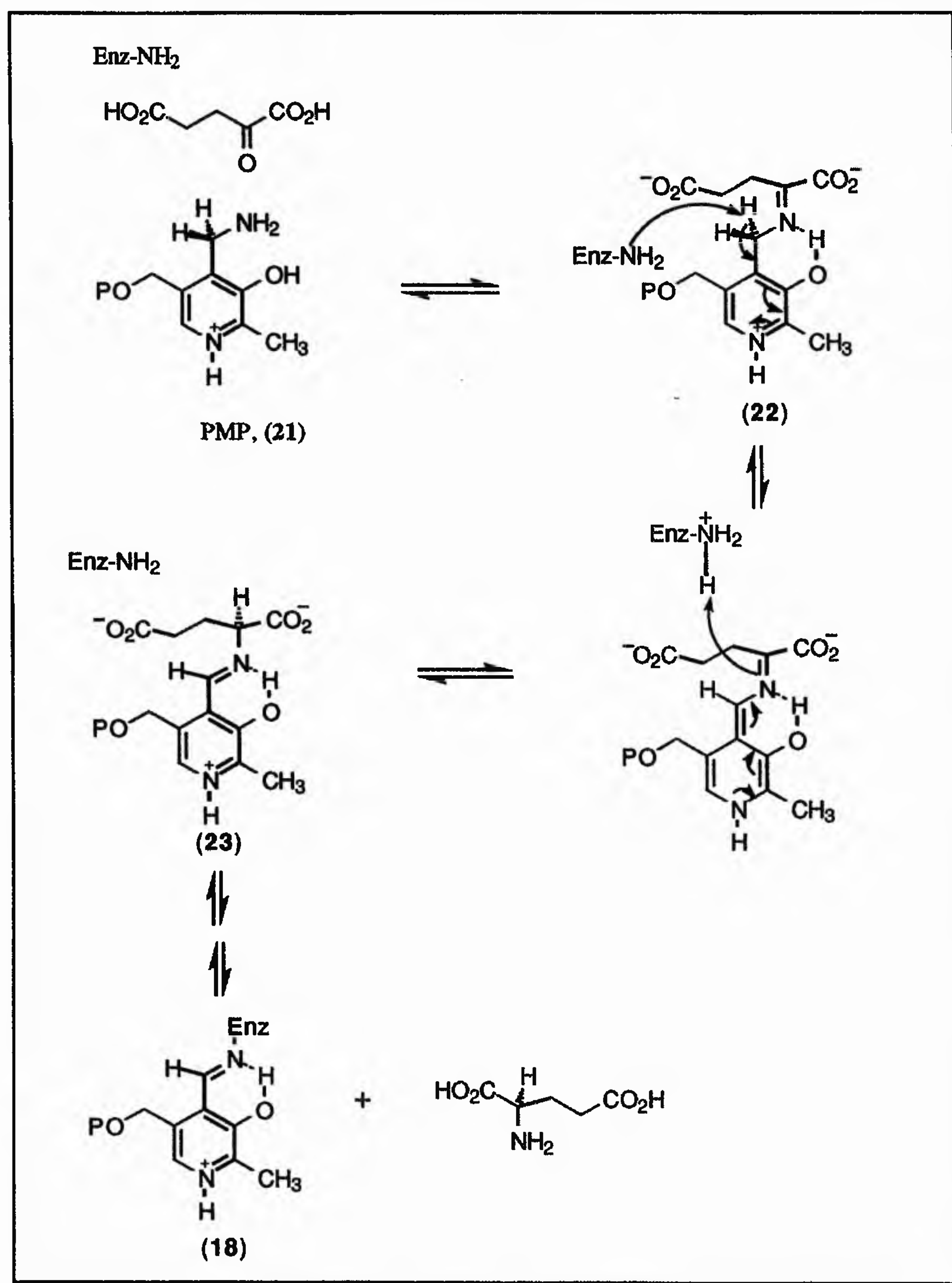
Transaminases (aminotransferases) are the best studied examples of of PLP-dependent enzymes.[21] They catalyse the interconversion of pairs of amino acids and α-keto acids in two half reactions.

In the first half reaction (Scheme 1.6a), the C^{α} proton of the aldimine **19** is transferred to the ketimine (**20**) at the C-4'-*Si* face to give the pyridoxamine (PMP, **21**) and an α-

keto acid (oxaloacetate). The second half of this transamination involves reaction of PMP (**21**) with another α-keto acid (e.g. α-keto glutarate) to give the ketimine (**22**). 1,3-Suprafacial proton transfer from the C-4'-*Si* face then gives the aldimine (**23**) which subsequently undergoes transaldimination to give a new (2*S*)-amino acid, (2*S*)-glutamate and the internal PLP-aldimine **18** (Scheme 1.6b).



Scheme 1.6a: *The first half reaction catalysed by aspartate aminotransferase.*



Scheme 1.6b: *The second half reaction catalysed by aspartate aminotransferase.*

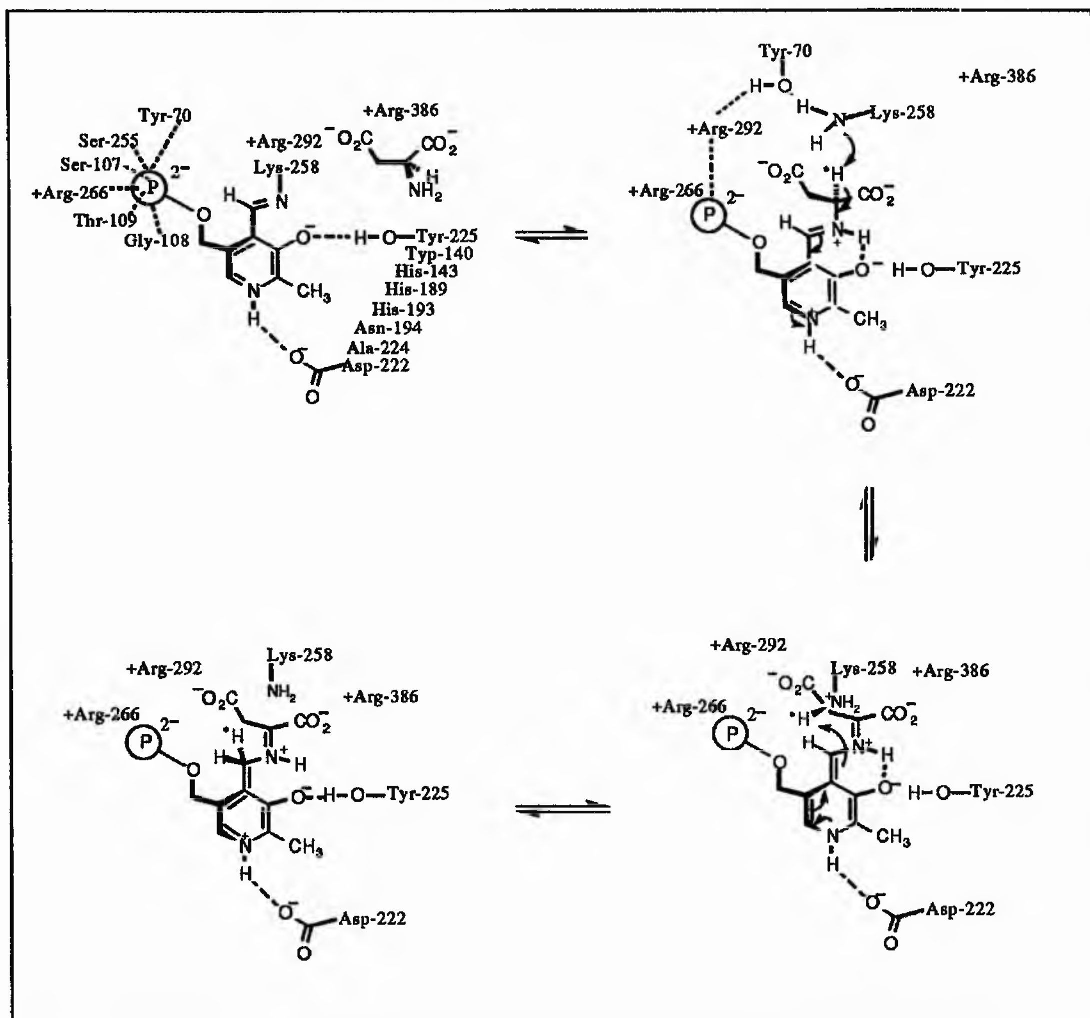
Aspartate aminotransferase (AAT) has received by far the most attention.[22-24] It catalyses the conversion of (2*S*)-aspartic acid and α-ketoglutaric acid to (2*S*)-glutamic acid and oxaloacetate. X-Ray structures of the active holoenzyme substrate complex have been published for chicken mitochondrial AAT,[25] pig cytosolic AAT,[26] and

chicken heart cytosolic AAT[27] at 2.8 Å. More recently the crystal structure of *E. coli* AAT has been determined,[28] and the overall folding of the protein was found to be similar to that of the mammalian enzymes.

Arigoni and Besmer,[18] and Dunathan *et al.*[19,29] independently studied the aminotransferase reaction. They discovered that although pyridoxal was a co-enzyme for the reaction, it did not bind to the apoenzyme as tightly as PLP. Both groups of researchers demonstrated that the removal of the proton from C-4' occurs from the 4'-*si*-face of the pyridoxamine. For the transamination catalysed by pyridoxamine pyruvate transaminase, it was shown that direct internal hydrogen transfer occurred between C^{α} of (2*S*)-alanine and C-4' of the co-enzyme suggesting that the protonation and deprotonation occur *via* a suprafacial mechanism mediated by a single base.

X-Ray crystallographic studies of mitochondrial chicken heart AAT identified the active site residues that are important for binding PLP and the substrate (Scheme 1.7). The protonated nitrogen of the pyridinium ring of the coenzyme forms a hydrogen bond with Asp-222, whilst the deprotonated phenolic hydroxyl is hydrogen bonded to the Tyr-225. The 2-methyl group lies in a pocket defined by eight amino acids. The 5'-phosphate ester is hydrogen bonded within a hydrophilic pocket to six amino acids, including Ser-255 and Arg-266. Arg-266 neutralises the dianionic phosphate. The proximal and distal carboxylate groups of the physiological substrates are bound by Arg-386 and Arg-292 respectively.

The acid-base mechanism proposed for aspartate aminotransferase suggests that L-aspartate binds with its α-amino group protonated, and that one of the α-amine protons is abstracted by the Schiff base between Lys-258 and the active site PLP.[30] It was further proposed that upon binding of the aspartate, the pKa value of its α-amino group decreases from a value of 9.8 to about 8, and the Schiff's base nitrogen pKa



Scheme 1.7: *Residues at the active site of chicken mitochondrial aspartate aminotransferase.*

value increases from 6 to 8.[31] The resulting matched thermodynamics between the α-amine and Schiff base nitrogen thus facilitates the initial proton transfer and thereby the overall rate of the aminotransferase reaction. Experimentally determined pKa values of the α-amino group of the aspartate and some of its analogues substituted at the α- and/or β-carboxylate and their calculated proton affinities, along with estimated proton affinities of complexes in which guanidinium is hydrogen-bonded to the carboxylates of aspartate, and for the L-aspartate Michaelis complex of aspartate aminotransferase agree with the above proposal. The decrease in the pKa value of the α-amine of aspartate has been proposed to result from the neutralization of the charge of its α-

and/or β-carboxylate.[31] The extent of charge neutralization that would result in the proposed pKa value decrease, and whether at the α- or β-carboxylates or both is not known. The interaction of the aspartate α- and β- carboxylates at the active site of aspartate aminotransferase is *via* hydrogen-bonds to Arg-292 and Arg-386.[32] These studies have improved the understanding of the conformational changes which occur in protein binding.[12]

Aspartate aminotransferase consists of two identical subunits each consisting of two domains. The co-enzyme is bound to the larger domain and is situated in an open pocket near the subunit interface which is made up of residues from both subunits. The two carboxylate groups of the dicarboxylic acid substrates are bound to Arg-386 and Arg-292 from adjacent subunits, and the substrate specificity is determined by these interactions. The stabilization provided by these interactions not only positions the substrate correctly for efficient catalysis but also shifts the conformational equilibrium to the closed form which brings Arg-386 3Å closer to the co-enzyme which is rigidly positioned in the smaller domain. Transaldimination of the ε-amino group of Lys-258 by the α-amino group of the substrate to form the external aldimine is accompanied by a tilting of the coenzyme by ~30°. The released ε-amino group of Lys-258 then serves as a proton donor/acceptor in the 1,3-prototropic shifts accompanying transamination. At this stage or after formation and hydrolysis of the initial ketimine, the co-enzyme rotates back by ~20°. Through out this process, the pyridinium ring is hydrogen bonded to the β-carboxylate group of Asp-222.

Two genetically distinct isoenzymes exist in animal tisssue, cytosolic (cAAT) and mitocondrial (mAAT).[33] The primary structures of a number of AAT's have been completely elucidated.[21] The amino acid sequence of mAAT's from chicken, pig, rat, mouse, horse and human and of cAAT's from chicken, pig, mouse and horse are known as well as those from *E. coli* B and *E. coli* K12.[34] In general the mAAT's from different sources show ~85% sequence identity.

Similiar identity is also found between cAAT's. However the sequence identity between two isozymes of the same species is only about 45%.[35] It should be noted that in all the above examples there is almost 100% identity for the region of the protein corresponding to substrate or co-enzyme binding sites. The active enzyme, as stated above is a dimer of two identical subunits (M_r 45000 Daltons)[36] and contains two independent active sites.[37,38] A comparison of the amino acid sequence for selected regions of AAT isoenzymes[39] and the amino acid sequences for the Schiff's base forming region of several AAT's[40] are shown in Table 1.2 and 1.3.

Table 1.2: *Comparison of the amino acid seqences for selected regions of AAT isozymes.*

Isoenzyme Source	Amino acid residue number									
	70	108	140	190	222	258	266	292	360	386
	*	*	*	*	*	*	*	*	*	*
Human (*mit.*)	EYL	SGTG	TWGNH	LHACAHNPTG	FFDMAYQGF	QSYAKN	ERN	IRP	MFC	GRI
Chicken (*mit.*)	EYL	SGTG	SWGNH	LHACAHNPTG	YFDMAYQGF	QSYAKN	ERA	IRP	MFC	GRI
Pig (*mit.*)	EYL	SGTG	SWGNH	LHACAHNPTG	FFDMAYQGF	QSYAKN	ERV	IRP	MFC	GRI
Rat (*mit.*)	EYL	SGTG	SGGNH	LHACAHNPTG	FFDMAYQGF	QSYAKN	ERV	IRP	MFC	GRI
Chiken (*cyt.*)	EYL	GGTG	TWENH	LHACAHNPTG	FFDSAYQGF	QSFSKN	ERV	VRT	MFS	GRI
Pig (*cyt.*)	EYL	GGTG	TWENH	LHACAHNPTG	FFDSAYQGF	QSFSKN	ERV	VRV	MFS	GRI
E. coli	NYL	GGTG	SWPNH	FHGCCHNPTG	LFDFAYQGF	SSYSKN	ERV	IRA	DFS	BRV

* Corresponds to the numbered residue, the residues are numbered according to the sequence for cytosolic pig AAT.

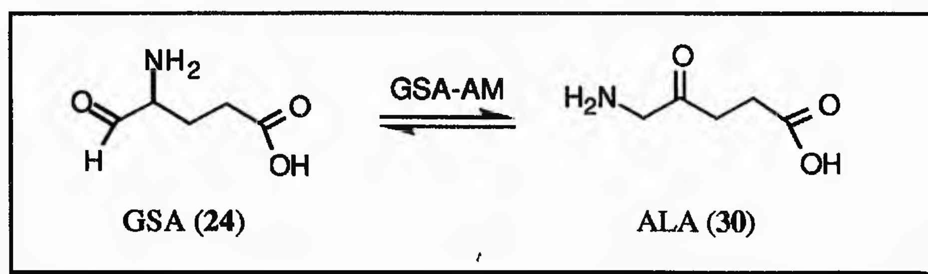
Table 1.3: *The Schiff's base forming regions of several isozymes.*

Isoenzyme Source	Amino acid source
E. coli	I V A S S Y S **K** N F G L Y
Chicken (*mit.*)	V L S Q S Y A **K** N M G L Y
Turkey (*mit.*)	V L S Q S Y A **K** N M G L Y
Pig (*mit.*)	C L C Q S Y A **K** N M G L Y
Rat (*mit.*)	C L C Q S Y A **K** N M G L Y
Human (*mit.*)	C L C Q S Y A **K** N M G L Y
Chicken (*cyt.*)	F C A Q S F S **K** N F G L Y
Pig (*cyt.*)	F C A Q S F S **K** N F G L Y

K-lysine which forms the Schiff's base

1.3.2 Glutamate 1-Semialdehyde Aminomutase

Glutamate 1-semialdehyde 2,1-aminomutase (GSA-AM) , EC 5.4.3.8[2] (glutamate 1-semialdehyde aminotransferase (GSA-AT)) is an exeptional enzyme among the transaminase because it catalyses the net transfer of the 2-amino group of glutamate 1-semialdehyde (GSA) **(24)** to the 1-position to give 5-aminolevulinic acid (ALA) **(30)**, Scheme 1.8.

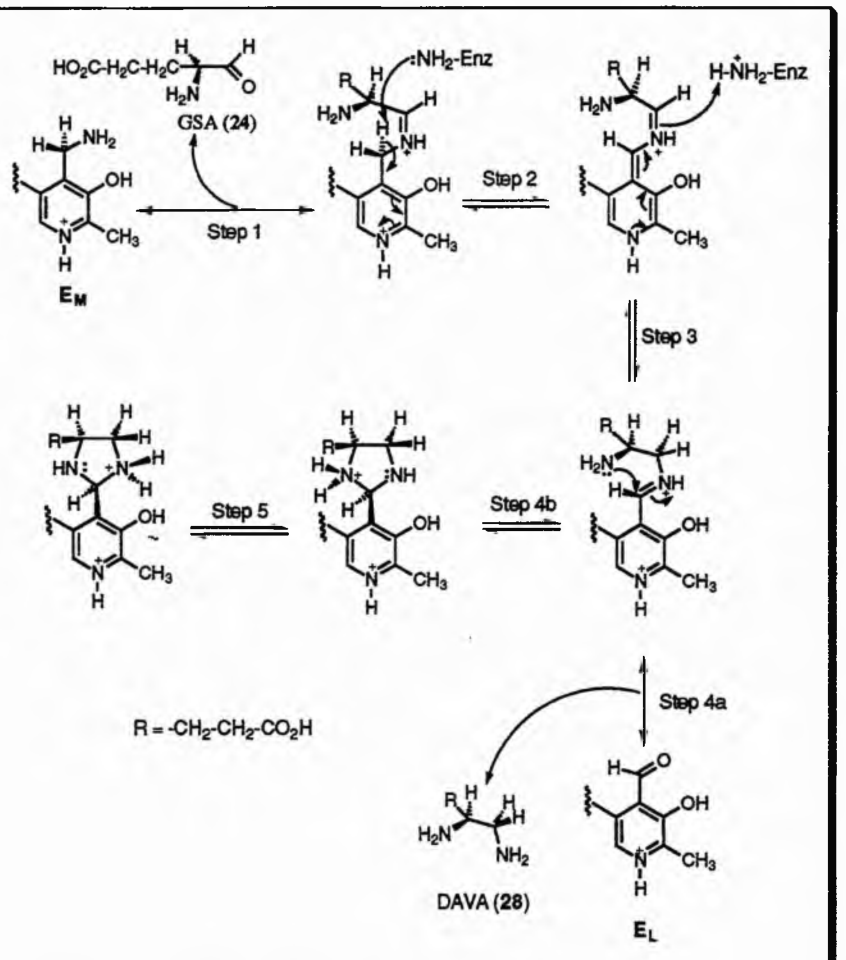


Scheme 1.8: *Reaction catalysed by glutamate 1-semialdehyde aminomutase.*

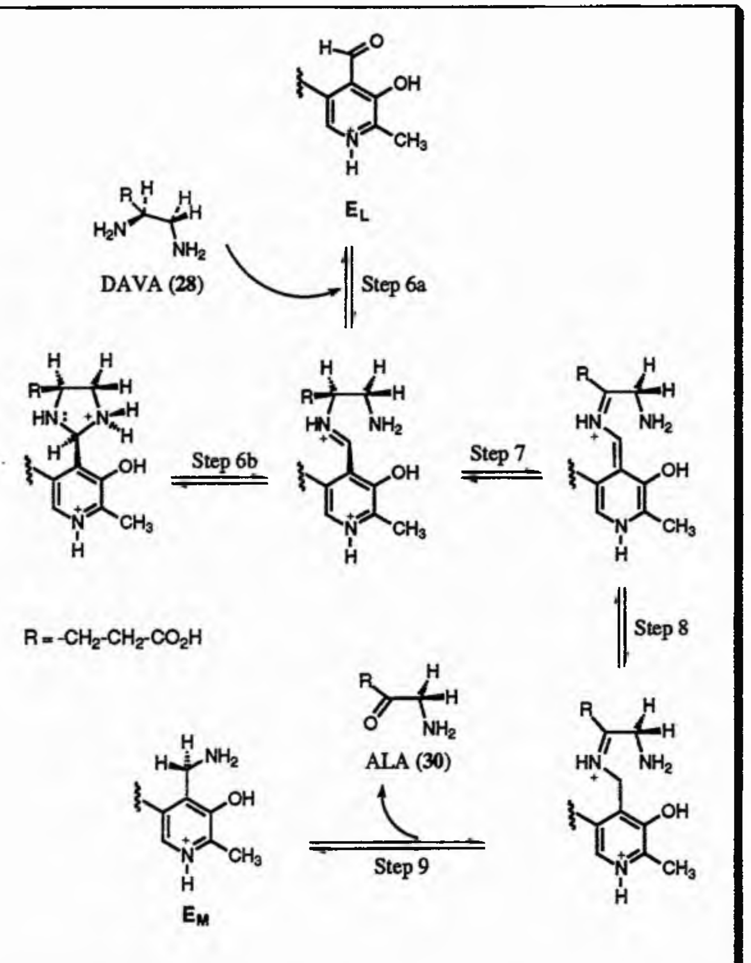
Although, the enzyme functions as a mutase, but based on structural,[41] spectroscopic[42] and steady state kinetic data[43] it can also belong to the family of aminotransferases and the mechanism for the amino group transfer is comparable to that of aspartate aminotransferase.

GSA-AM was first isolated from the chloroplast stromal fraction of greening barley and later from *Chlamydomonas* and *Synechococcus*. The enzyme is a dimeric protein consisting of two identical subunits of 46 kDa in barley and *Synechococcus*,[44] while in *Chlamydomonas* [45] the active form of the enzyme is 43 kDa.

As in all other aminotransferases, it is generally accepted that PLP is covalently bound to a lysine residue as an internal aldimine at the active-site. Purified GSA aminomutase is primarily in the pyridoxamine-P form. Both the pyridoxamine-P and pyridoxal-P form are able to react with GSA. The resulting intermediates are presumed to be 4,5-diaminovaleric acid (DAVA) (**28**) and 4,5-dioxovaleric acid (**31**) respectively. A likely mechanism proposed by Smith *et al.*[46] is shown in Schemes 1.9a & 1.9b.



Scheme 1.9a: *The first half reaction catalysed by glutamate 1-semialdehyde aminomutase.*

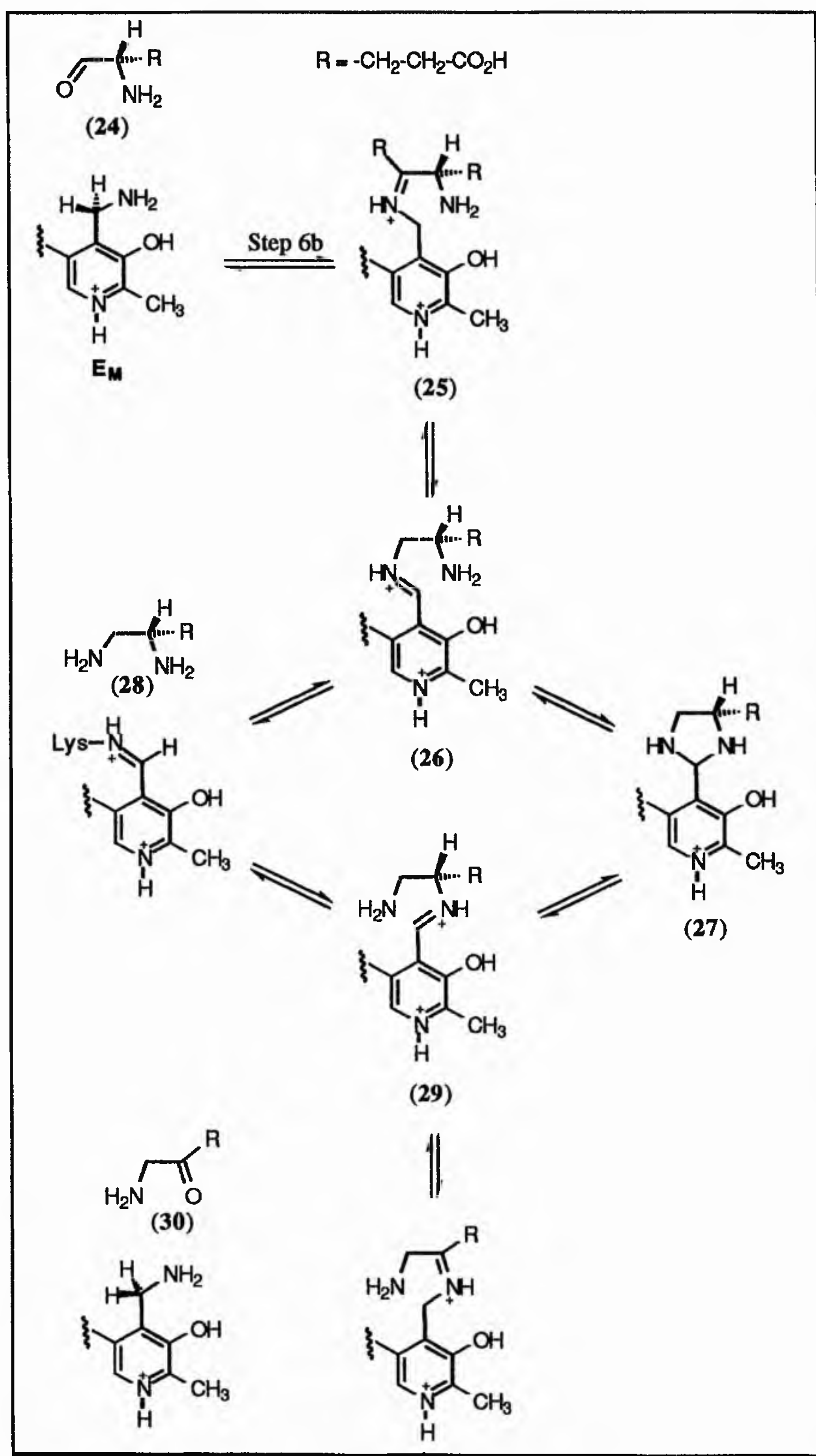


Scheme 1.9b: *The second half reaction catalysed by glutamate 1-semialdehyde aminomutase.*

The reaction is initiated with GSA and the pyridoxamine-P form of the enzyme (E_M), resulting in the formation of the C-1 aldimine of (S)-GSA (step 1, Scheme 1.9a). Abstraction of a methylene hydrogen from the C-4'-*si* face of the coenzyme (step 2) gives the corresponding quinoid intermediate, which upon proton transfer to C-1 is converted to the external aldimine of enzyme-bound pyridoxal-P (step 3). This intermediate undergoes transaldimination by the amino group of the active site Lys-272 (step 4a) or by the C-2 amino group of GSA (step 4b), to give (*S*)-DAVA and the pyridoxal co-enzyme form of GSA-AM (E_L), or, the corresponding cyclic imidazolidine adduct of pyridoxal-P, respectively.

The second half reaction (step 6-9, Scheme 1.9b) is similar except that the chiral centre at C-2 rather than the C-1 carbonyl of GSA is directly involved in amino group transfer, and the steps occur in the reverse order. Prototropic rearrangement (from C-2 of GSA or C-4 of DAVA to the C-4'-methylene, step 7 and 8) is also from the C-4'-*si* face of the original quinoid intermediate. During this stage of the reaction (step 6-9), the C-2 chiral center is directly involved in amino group transfer. C-4 hydrogen DAVA is on the C-4'-*re* face of the original quinoid intermediate (step 6), prototropic rearrangement is very unlikely. By analogy with aspartate aminotransferase, proton transfer from this side apparently does not occur at appreciable rates.

Pugh *et al.*[47] on the other hand concluded that only the pyridoxamine form of the enzyme is active in catalysing the conversion of glutamate semialdehyde to aminolevulinate and that the catalytic mechanism includes enzyme-bound diaminovalerate as a central intermediate. Both the groups of Smith and Pugh however, agree that the major route for the catalysis takes place through the initial combination of the enzyme in its pyridoxamine form (E_M) with the aldehyde group of the substrate and that the reaction proceeds through interconversion of tautomers (**26** and **29** in Scheme 1.10) of the imine of diaminovalerate with the pyridoxaldimine phosphate form of the enzyme (E_L).



Scheme 1.10: *A possible mechanism for the conversion of of glutamate 1-semialdehyde (24) into aminolevulinate (30).*

On the other, both Pugh *et al.*[46] and Smith *et al.*[47] differ in their opinion of the role and activity of E_L and in the requirement for release of the intermediate, diaminovalerate. Pugh *et al.* concluded that E_L did not contribute significantly to the catalysis of the reaction since reduction of E_L with $NaBH_4$ produced no detectable loss of enzyme activity. Conversely Smith *et al.*[43] concluded that the enzyme has one third of the activity of E_M. Recently Brody *et al.*[48] showed that GSA-AM absorption at 338 nm is due to pyridoxamine phosphate, while absorption at 418 is due to pyridoxal phosphate bound to Lys-272 by a Schiff's base.

1.3.3 Racemization

Amino acid racemases catalyse the interconversation of (*S*)- and (*R*)-amino acids.[6] They allow the deprotonation of the substrate aldimine and subsequent protonation of the ketimine to occur from opposite faces of the co-enzyme. Racemases can catalyse racemization by using two enzyme-bound bases, one on each face of the co-enzyme (Figure 1.1), or one base as depicted in Scheme 1.11. The one-base system requires the protein to undergo large changes in conformation so that each face of the co-enzyme is accessible. This is known as the 'swinging door' mechanism.[49]

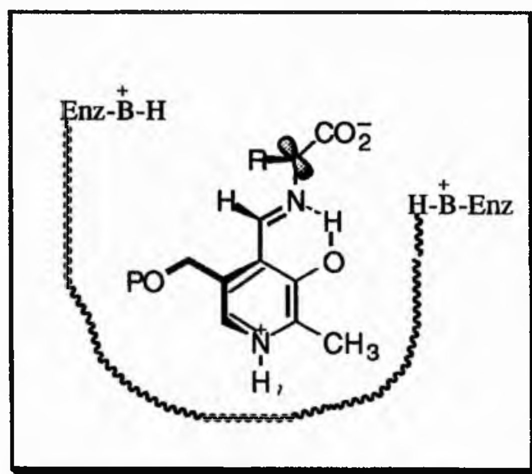
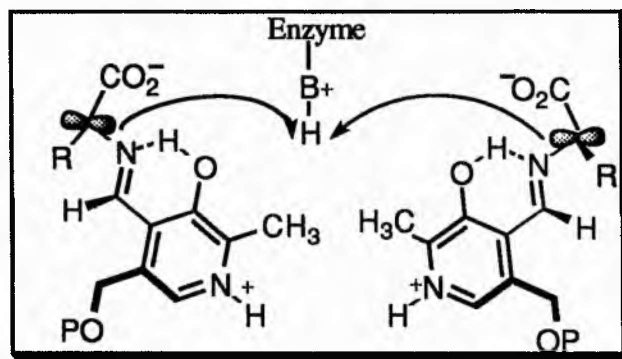


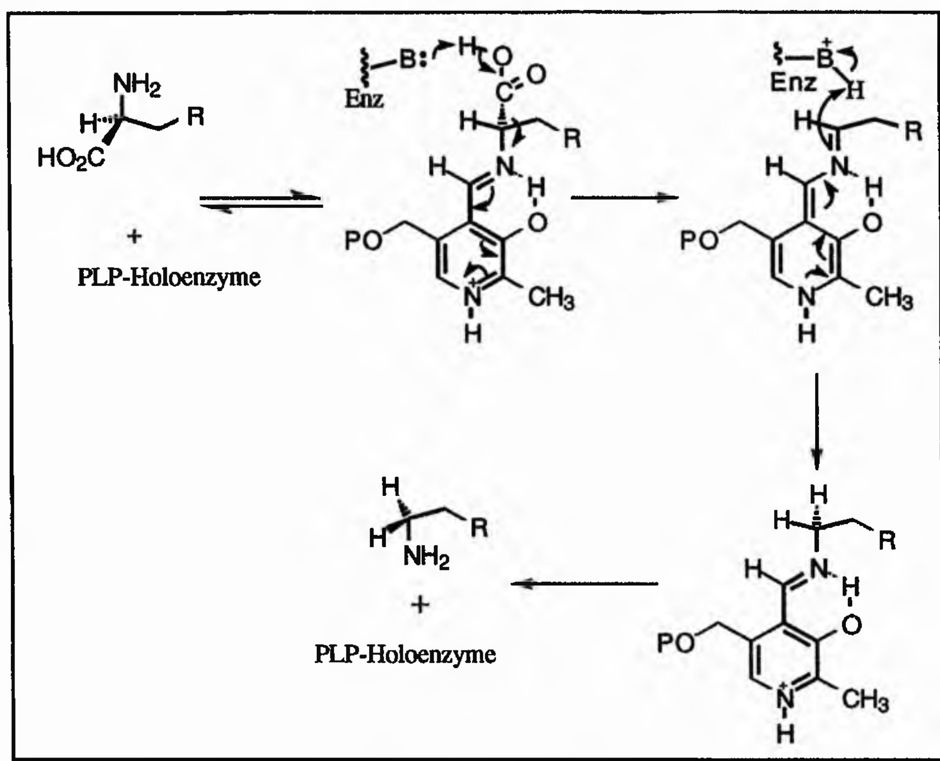
Figure 1.1: *Two enzyme-bound base racemization.*



Scheme 1.11: *One enzyme-bound base racemization.*

1.3.4 Decarboxylation

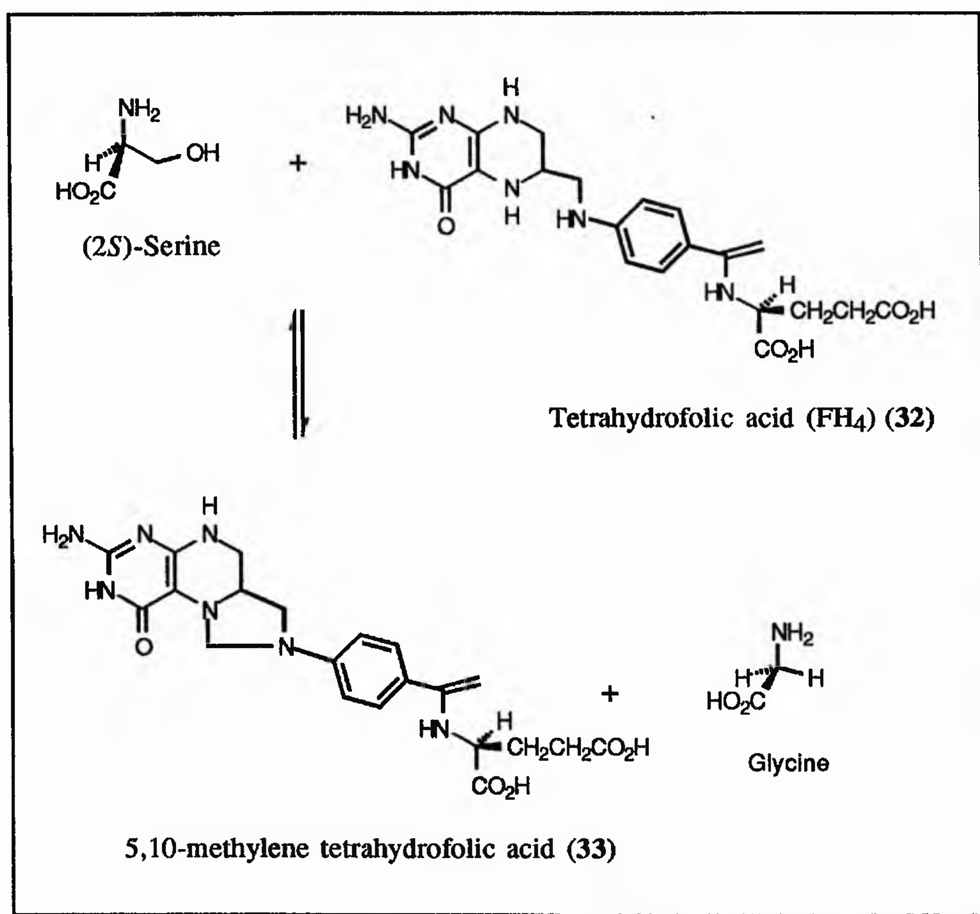
Specific decarboxylases are known for more than ten amino acids. In each case the α-carboxyl group is cleaved to give CO_2 and an amine as the products (Scheme 1.12). The N-C^{α} bond is disposed so that the carboxyl group of (2*S*)-amino acid is perpendicular to the conjugated π-electron system, presumably on the 4'-*si* face of the co-enzyme. To date, with the exception of three enzymes, the steric course of all reactions of α-amino acid decarboxylases examined from plant, microbial, and mammalian sources acting on L-amino acids has been shown to be retentive. These include glutamate,[50-52] histidine,[53,54] tyrosine,[55] lysine,[56] ornithine,[57-58] arginine,[58,59] and methionine decarboxylase.[59] The three decarboxylases which show alternative stereochemical courses are aminomalonate decarboxylase (an activity of serine hydroxy-methyltransferase (SHMT)),[60] which produces racemic labelled glycine, and wheat[61] and batcterial[62] α,ω-meso-diaminopimelic acid decarboxylases which decarboxylate at the (*R*)-amino acid centre of the substrate to give (2*S*)-lysine.



Scheme 1.12: *A general mechanism for decarboxylation.*

1.3.5 Cα-R bond cleavage or formation

The most intensively studied enzyme of this class is serine hydroxymethyltransferase, which catalyses the formation of 5,10-methylenetetrahydrofolic acid (**33**) and glycine from tetrahydrofolic acid (**32**) and (2*S*)-serine by β-carbon cleavage and is thus a key enzyme in single carbon (C_1) metabolism at all oxidation levels (Scheme 1.13). In the absence of tetrahydrofolic acid, a slower reaction occurs, giving glycine and formaldehyde. SHMT not only catalyses the retro aldol cleavage of a number of substrates to give glycine and the appropriate aldehydes[63-67] but also decarboxylate aminomalonic acid[68], transaminates (2*S*)-alanine[69-71] and catalyse α-hydrogen exchange and aldol condensation with (2*S*)-amino acids.[72] The exceptional range of catalytic activity shown by SHMT has stimulated mechanistic investigations into each of these reactions.[73]

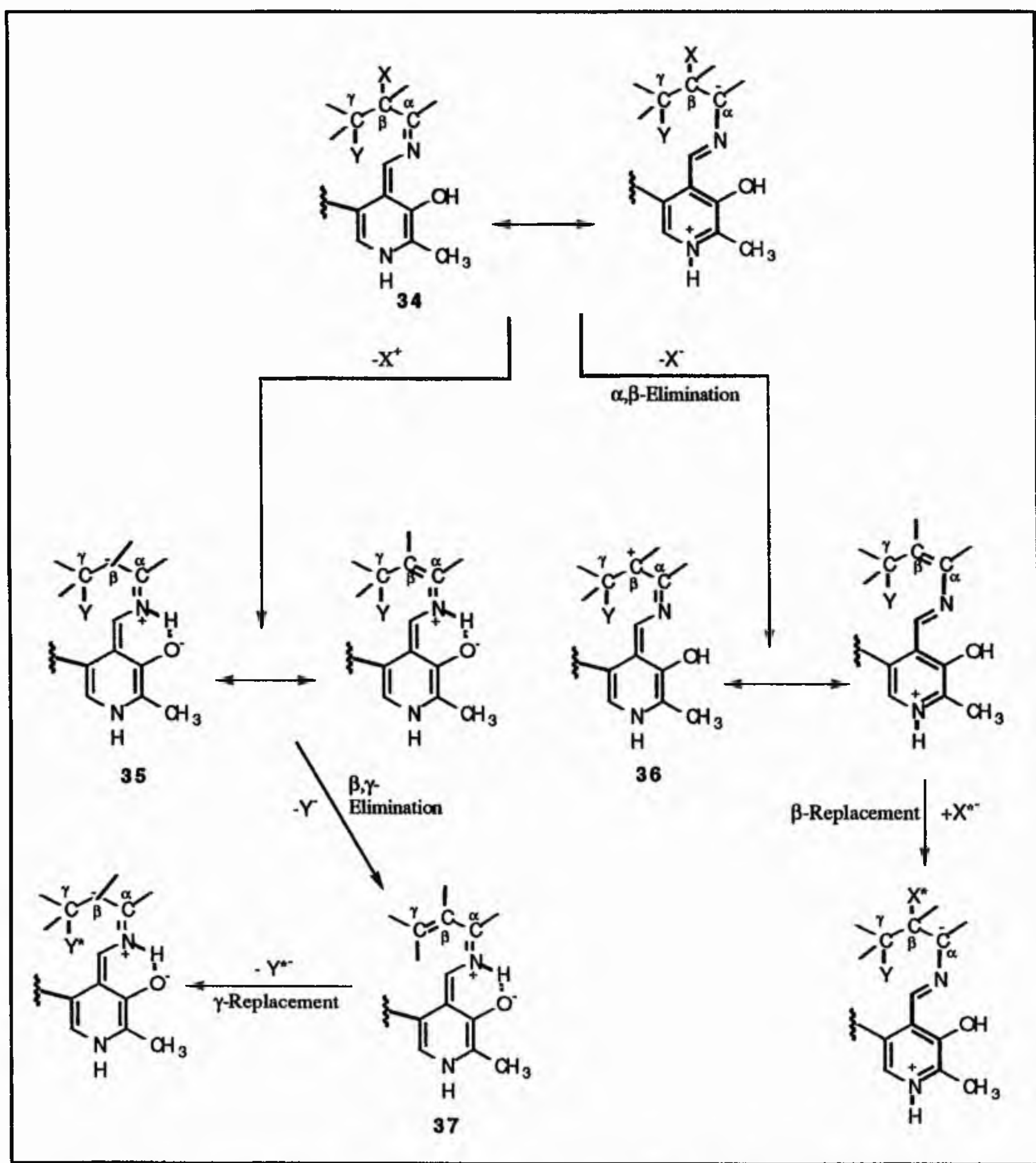


Scheme 1.13: *The physiological reaction catalysed by SHMT.*

1.4 Reactions at the β- and γ–Carbon

A large group of PLP-dependent enzymes catalyse nucleophilic displacement of a substituent at the β-position of amino acids. These β-replacement reactions involve initial cleavage of the C^{α}-H bond of the PLP-substrate imine to generate a quinoid intermediate, followed by elimination from C-β of an anionic group X^{-}, or of HX (after protonation of X, thus making it a better leaving group), (Scheme 1.14).[74] The resulting amino acrylate intermediates **35** can undergo the reverse reaction sequence, Michael addition of X'^{-} and H^{+} at C^{β} and C^{α} to generate the PLP-product imine, representing the β-replacement reaction. Alternatively, hydrolysis of **35** to pyruvate

and ammonia constitutes the α,β-elimination reaction. To date all of these β-replacement and α,β-elimination-deamination reactions have been shown to occur in a retentive mode.



Scheme 1.14: *PLP-catalysed reactions at C-3 and C-4 amino acids.*

Several PLP-dependent enzymes also catalyse β,γ-elimination and γ-replacement reactions.[75] Mechanistically, the sequence involves deprotonation at the α and β carbons, followed by elimination of an anionic group Y from C^{γ}, to yield the key PLP-

vinylglycine intermediate (Structure **37**, Scheme 1.14). In γ-replacement reactions, nucleophilic attack by Y' on C^{γ} precedes reprotonation at C^{β} and C^{α} as in the cystathionine γ-synthase reaction. Alternatively, protonation at C^{γ} generates a PLP-α-aminoacronate Schiff base, equivalent to the aminoacrylate intermediate of β-replacement and α,β-elimination reactions. Substitution can occur at C^{β} by OH (threonine synthase) to give α,β-substituted amino acid or by H^+ (γ-cystathionase) to initiate hydrolysis to a α-ketobutyric acid.

1.5 Suicide inhibitors of PLP dependent enzymes

In general, PLP-dependent enzymes are inhibited by carbonyl-attacking reagents, for example, hydroxylamines and hydrazines, and by substrate analogues which cannot undergo reaction, but which contain essential active-site binding groups. In the absence of the endogeneous co-enzyme, some PLP-dependent enzymes are inhibited by the production of PMP in an abortive transaminase side reaction. In inhibitor design, some effort has been directed towards the synthesis of substrate analogues containing carbonyl-attacking groups, for example, hydrazino acid[76] and groups that prevent initial deprotonation, for example, C^{α}-methyl amino acids.[77] However, most effort has focused upon the design of mechanism-based (k_{cat}) inhibitors.[76-80] These type of compounds are non reactive until activated by the holoenzyme.

There has been a wide interest in these inhibitors as potential drug candidates, because of the pharmacological and therapeutic utility of inhibiting specific PLP enzymes (Table 1.3).

Enzymes that require PLP, catalyse some chemical change at the α,β or γ-carbon of the common α,β or γ-amino acids. In every case the role of the PLP moiety is to stabilise

the carbanionic intermediates that develop during the catalytic process. The suicide inhibitors all have latent functional groups that become activated by their proximity to

Table 1.3. *Some therapeutically important target enzymes dependent on PLP.*

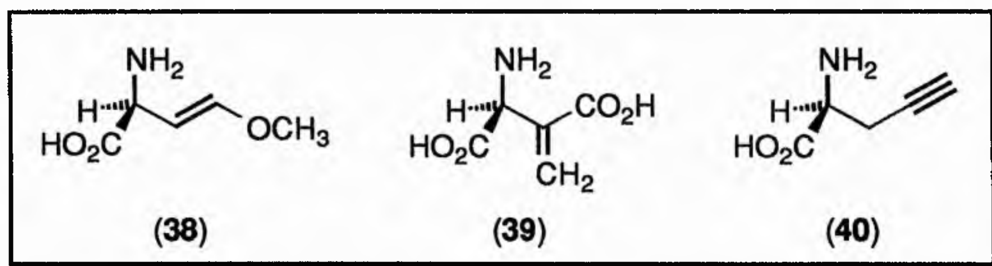
Enzyme	Potential effect
bacterial alanine racemase	antibacterial
brain GABA transaminase	antiepileptic
mammalian DOPA decarboxylase	antihypertensive
mammalian ornithine decarboxylase	antineoplastic

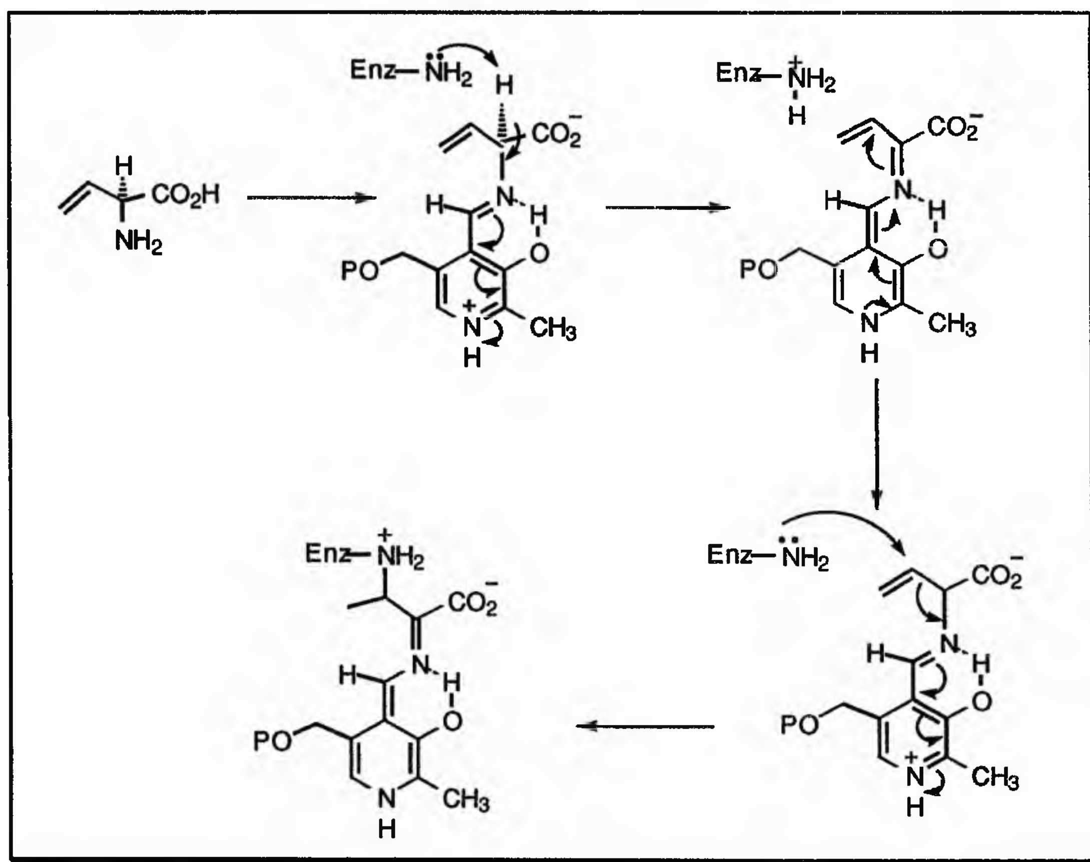
the site of an enzyme-generated carbanion. Such an intermediate can break down to yield a reactive species that may react with an active site amino acid side chain, or with tightly bound PLP co-enzyme. Each of these processes leads to the inactivation of the enzyme, in the first case the active site is blocked by an unreactive species and in the second case the co-enzyme is no longer available to bind to the substrate. Mechanism based inactivating fuctional groups that inhibit PLP enzymes include, acetylinic, olefinic, β-halo substituents and other leaving groups, nitriles, aryl sulphoxides, dihydroaromatics and phosphonoamino acids.[79]

1.5.1 Transaminase Inhibitors

In transamination reactions there are two half reactions for a complete catalylic cycle (see Section 1.3.1). In the first half reaction catalysis proceeds through the substrate-PLP anion, and this intermediate as well as the product α-imino-PMP enzyme complex (Scheme 7a) are species allowing for activation of latent functional groups in suicide substrates. Activation by net oxidation to the ketoacid equivalent is observed in both olefinic and acetylinic amino acid analogues. The olefin analogues include vinyl

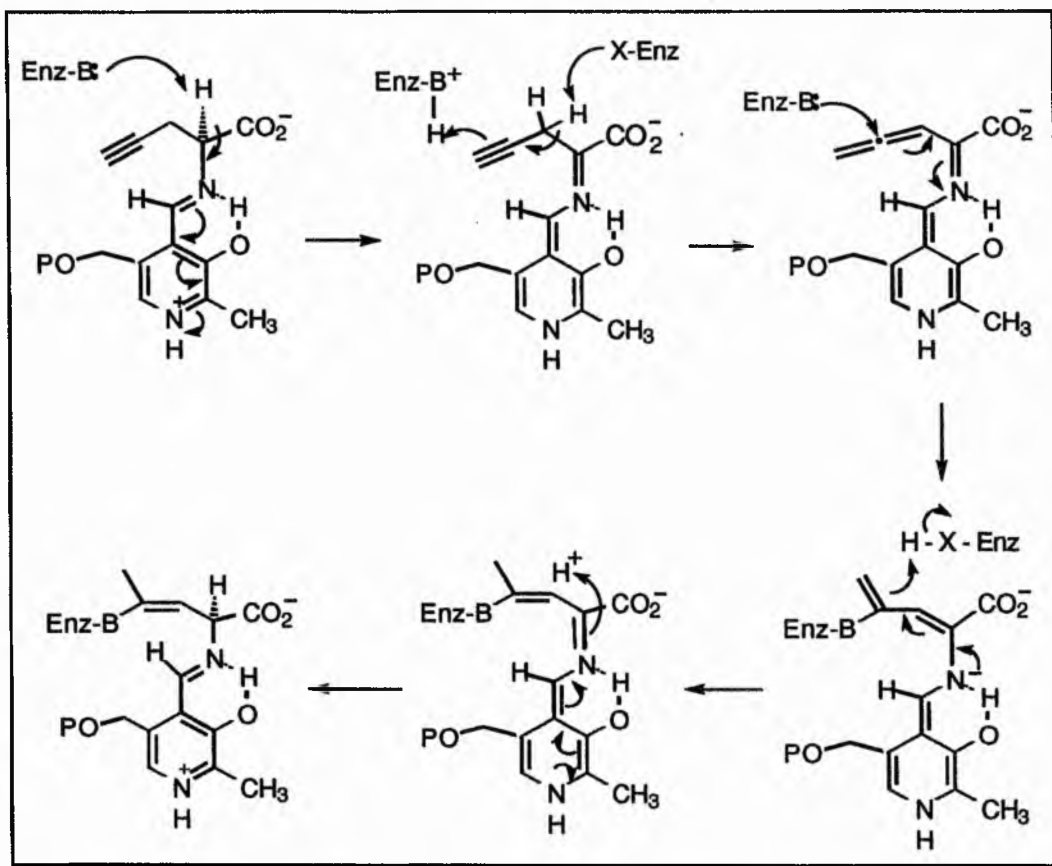
glycine (**38**),[81,82] β-methylene aspartate (**39**)[83] and (E)-methoxy-vinyl glycine (**40**)[84] and the generally accepted inactivation mechanism is as outlined in Scheme 1.15.





Scheme 1.15: *Inactivation of aspartate aminotransferase by vinyl glycine.*

The acetylenic analogues include the natural product (2*S*)-propargylglycine,[85] and the mechanism of inactivation is depicted in Scheme 1.16.

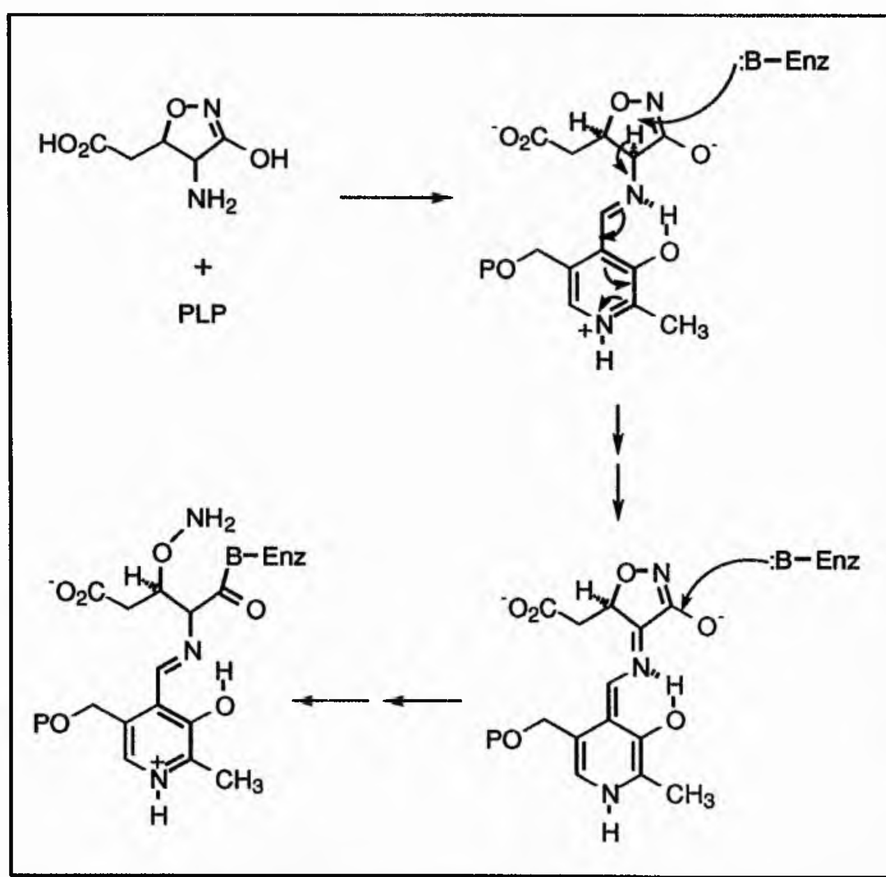


Scheme 1.16: *Inactivation of AAT by propargylglycine.*

Some of the more common types of inhibitors contain good leaving groups at C^{β}. These compounds are able to undergo facile elimination to generate olefinic intermediates. These include, 3-chloro-(2*S*)-alanine,[86,87] an inhibitor of alanine aminotransferase, and (2*S*)-serine O-sulfate[88] which inhibits aspartate aminotransferase and glutamate decarboxylase.

The accepted mechanism had been that the 'killing' species was the aminoacryl-PLP intermediate, which undergoes a putative Michael reaction with an enzyme bound nucleophile. Although this is the case with some enzymes, such as serine hydroxy methyl-transferase (SHMT), Metzler and co-worker[89] have shown that the inactivation of aspartate β-decarboxylase is not due to the occurrence of a Michael reaction. Bright

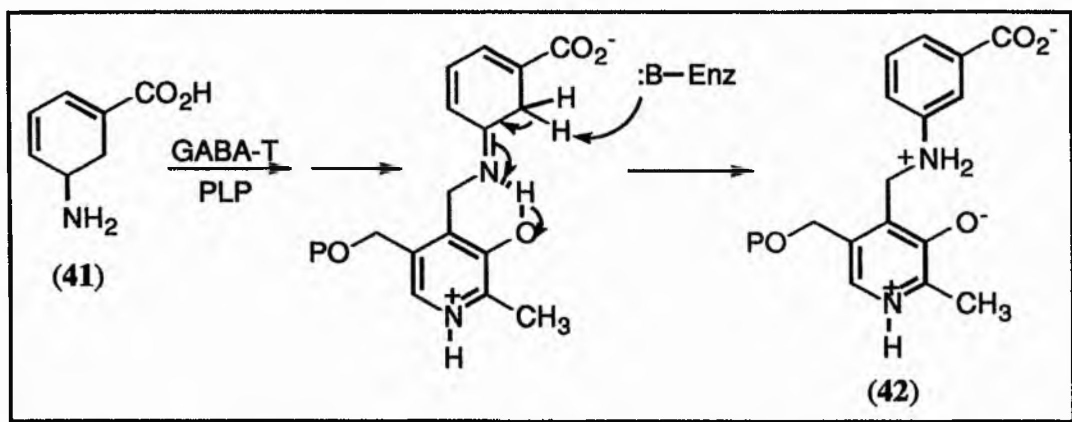
and co-workers have used the nitro group of 3-nitro alanine as an inactivator of aspartate aminotransferase and alanine aminotransferase.[90] The nitro alanine undergoes α-proton abstraction followed by β-nitro elimination. Alanine aminotransferase is also inhibited by cycloserine,[91] while aspartate aminotransferase is inhibited to varying extents by α and γ-cycloglutamic acids[92-94] (Scheme 1.17). α-Cycloglutamic acids is believed to acylate an active site bound nucleophile to give a stable inactivated complex, whilst γ-cycloglutamate is thought to form an oxime of β-aminooxyglutamate with PLP by ring opening of the isoxazolidine ring.



Scheme 1.17: *Inactivation of AAT by cycloglutamate.*

Various analogues of GABA inhibit GABA-transaminase. These include β-chloro-, 3-phenyl, γ-acetylenic and γ-vinyl GABA as well as ethanolamine *O*-sulfate.[95] Mammalian GABA-transaminase is inhibited by the naturally occurring product

gabaculine (**41**) *via* an interesting aromatisation mechanism to give the conjugated PMP adduct (Scheme 1.18, (**42**)).[96-97]



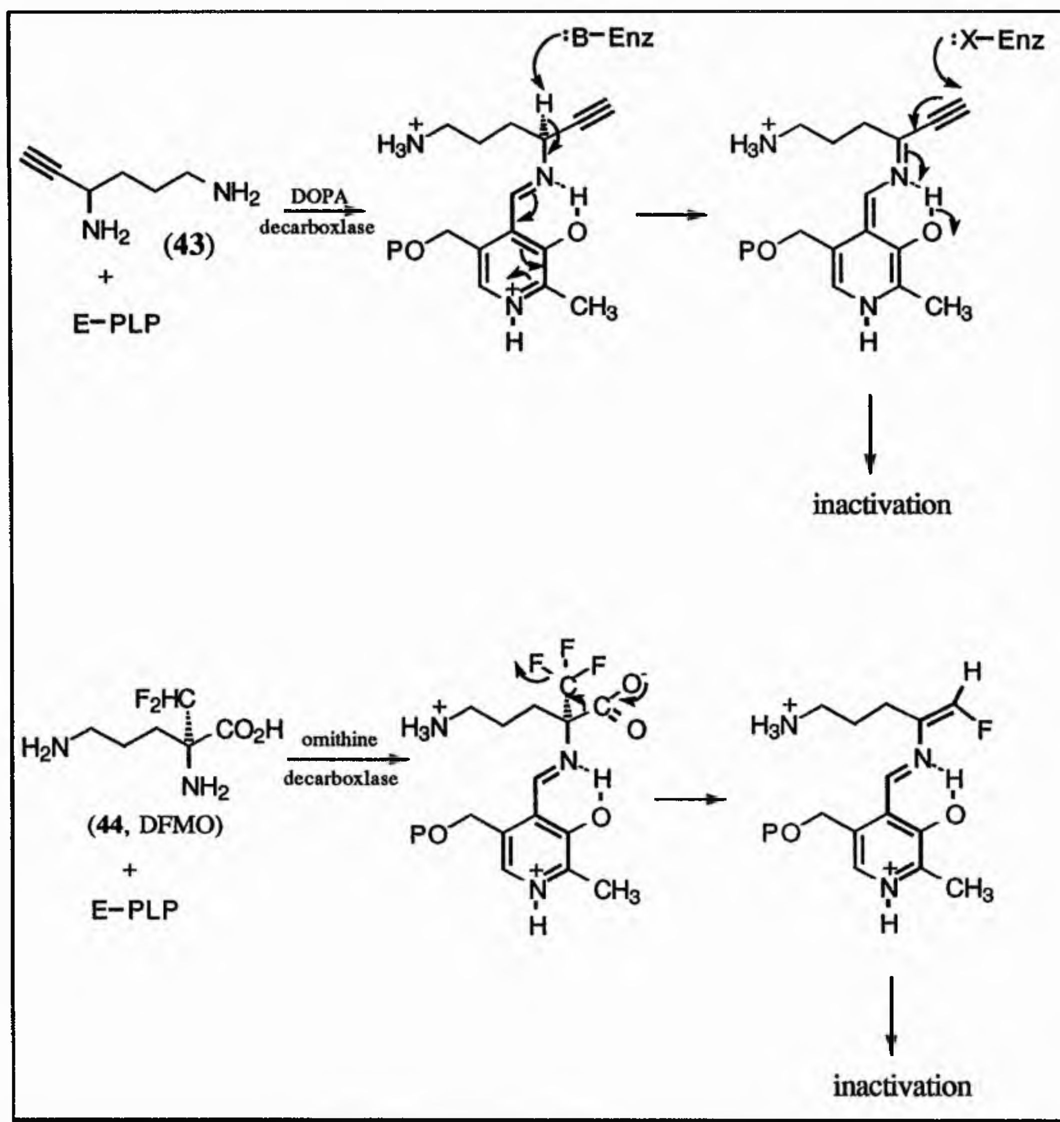
Scheme 1.18: *Mechanism of inactivation of GABA-transaminase by gabaculine (41)*

PLP dependent racemase enzymes are important because they are involved in the biosynthetic pathway leading to peptidoglycan. Many suicide substrates are known for the racemases, including β-substituted alanines for alanine racemase[98-100] and phosphoalanine (for gram positive alanine racemase only).[101-102] Phosphoalanine is thought to inhibit alanine racemase *via* an initial reversible formation of a weak complex which slowly isomerises to a stoichiometric complex that dissociates extremely slowly. The complex is not reducible with borohydride.[103]

1.5.2 Other PLP Inhibitors

Decarboxylase inhibitors are particularly important because of the role these enzymes play in the biosynthesis of many pharmocologically active amines (see p. 23). Many examples with alkyne, olfenic, and mon-, di or trihalomethyl groups have been reported. Since decarboxylases do not abstract the substrate α-hydrogen they can decarboxylate α-methyl amino acids. Thus α-ethynyl, α-vinyl, α-fluoromethyl, and

α,α-difluoromethyl amino acids serve as sucicde substrates. For example, the inactivation of DOPA decarboxylase by α-vinyl DOPA[104-105] (**43**) and of ornithine decarboxylase by α,α-difluoromethylornithine[106] (**44**) are shown below:

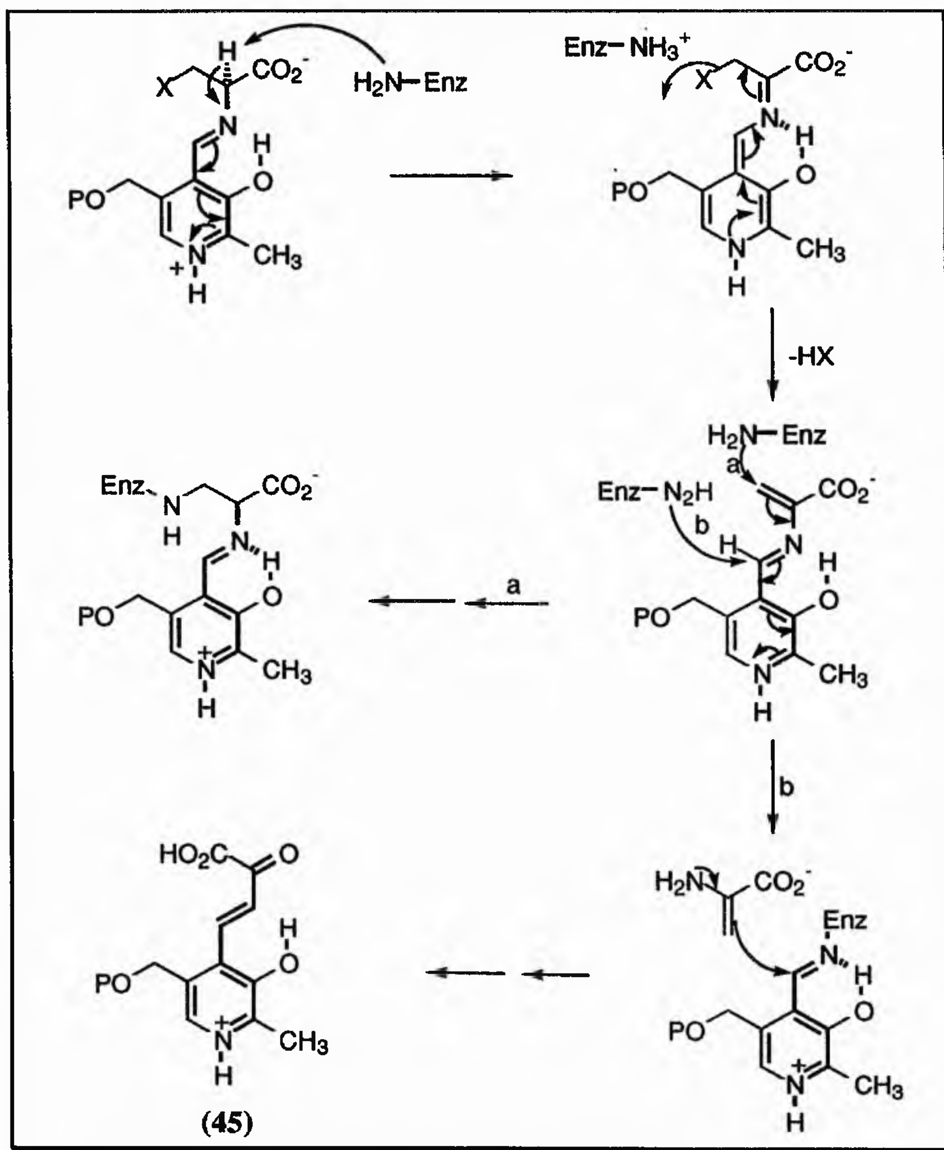


Scheme 1.19: *Inactivation of DOPA decarboxylase by α-vinyl DOPA (43) and of ornithine decarboxylase by α,α-difluoromethylornithine (44).*

5-Hexyne-1,4-diamine inactivates ornithine decarboxylase,[107] presumably *via* similar mechanism to that described for α-vinyl DOPA. Other inhibitors of ornithine decarboxlyase include β, γ-dehydroornithine,[108] α-fluoromethyl putrescine[109] and α,α-difluoromethyl ornithine.[107] Halomethyl and olefinic groups have been

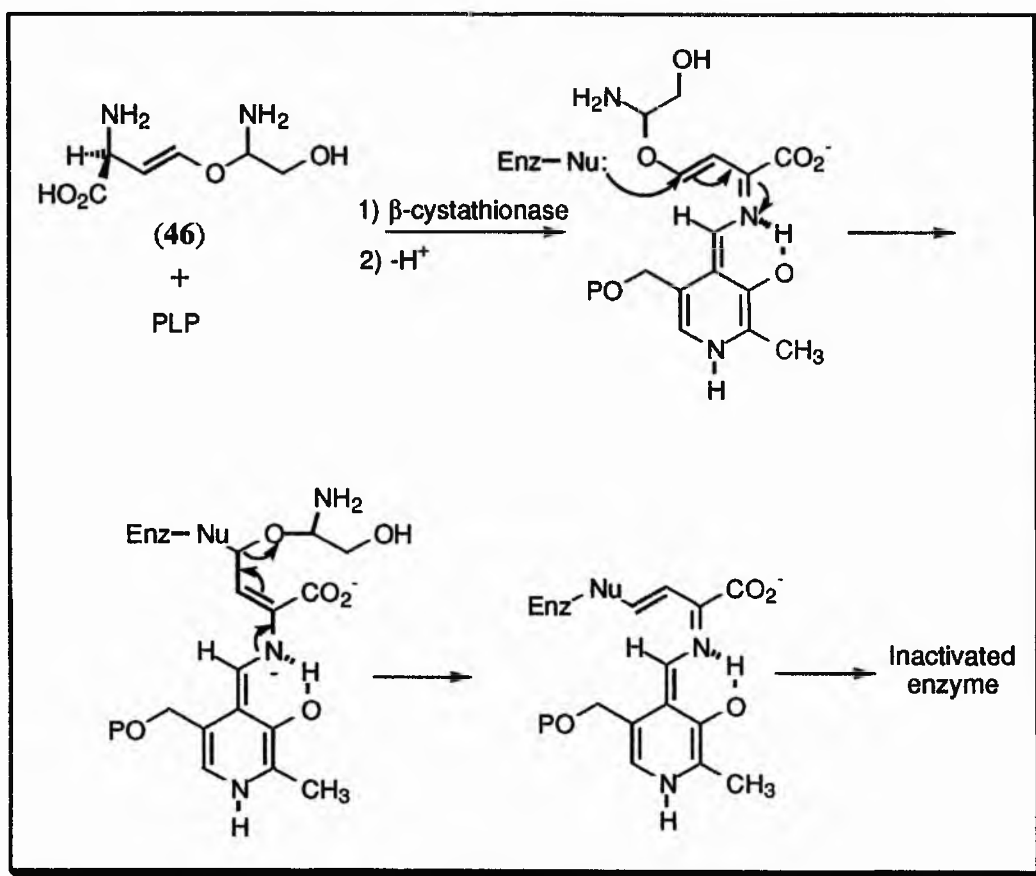
incorporated into a single molecule by Bey and collegues.[110] α-Fluoromethyldehydroornithine and α,α-fluromethyldehydroputrescine have been tested as ornithine inhibitors. The dehyroornithine is a more effective inhibitor than the saturated analogue and its K_i is 30 times lower.

Enzymes which catalyse α,β-elimination occasionally undergo an abortive reaction with a physiological substrate. For example, threonine deaminase is very slowly inactivated by (2*S*)-serine approximately once every 10^4 turnovers,[111] note that a similar situation occurs with 3-chloroalanine.[112] In contrast, (2*S*,3*R*)-threonine does not cause inactivation, presumably as the intermediate formed is less susceptible to Michael addition or enamine condensation to give the Schnackerz type product (**45**) (Scheme 1.20).[113]



Scheme 1.20: *Two possible mechanisms for the inactivation of enzymes that catalyse α,β–elimination reactions. Route a causes the modified coenzyme to be covalently bound to the enzyme, and route b leads to the Schnackerz adduct (**45**).*

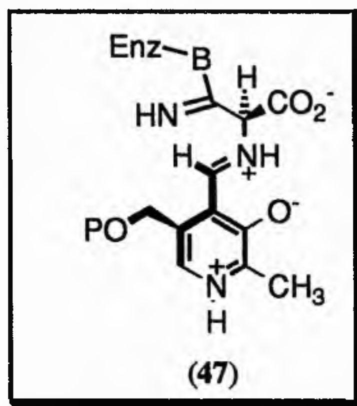
E. coli tryptophanase is irreversibly inhibited by trifluoroalanine.[114] It is proposed that the difluoroaminoacrylyl-PLP-aldimine complex is more susceptible to nucleophilic attack at C-3 by an enzyme bound base, than to hydrolysis at the aldimine carbon atom. The enzyme β-cystathionase is irreversibly inactivated by the fungal toxin rhizobiotoxin (Scheme 1.21 (**46**)).[115] The alkoxyvinylglycine analogue rhizobiotoxin may undergo initial C^{α}-H abstraction, and then an addition and elimination sequence, similar to that for (*E*)-methoxyvinylglycine (**40**) with aspartate aminotransferase.



Scheme 1.21: *The inactivation of β-cystathionase by rhizobiotoxin (**46**).*

β-Cystathionase is also irreversibly inhibited by trifluoroalanine, and tryptophan synthase is subject to suicide inhibition by trifluoro-, trichloro- and dichloroalanine.[116] *E. coli* tryptophan synthase also reacts with cyanoglycine, causing inhibition which is

reversible upon dialysis or gel-filtration. The inactivated coenzyme is presumed to be a stable β-imine PLP adduct (**47**).[117]



(**47**)

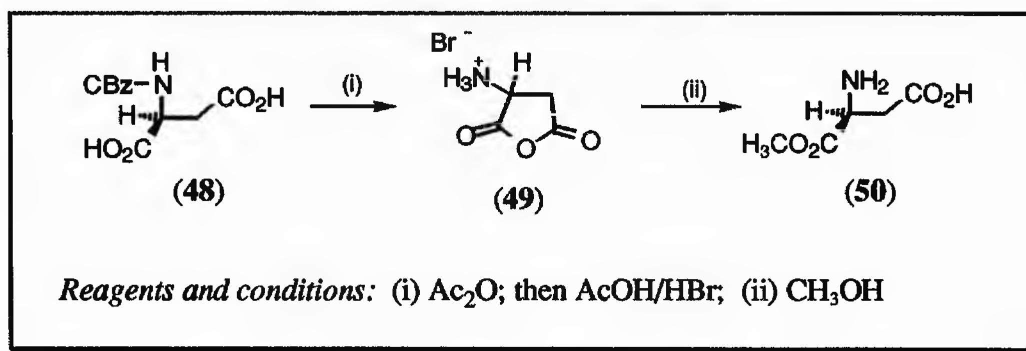
Chapter Two

RESULTS AND DISCUSSION

2.0 Results and Discussion

2.1 Synthesis of aspartic acid methyl ester and amide derivatives

J. Kovacs *et al*[118] have reported on the formation of α-methyl aspartate ester (**50**) from five membered ring aspartic anhydride hydrobromide (**49**) (Scheme 2.1). Due to the strong inductive effect of the protonated amino group, the anhydride ring was opened predominantly in the α-position. An excess of silver oxide was needed to remove the bromide ion.[119] Electrophoretic[120] and chromatographic analysis of the mixture of asparagine and isoasparagine (obtained from the reaction of the crude ester with ammonia) indicated that the α-methyl ester was obtained in 95% yield.

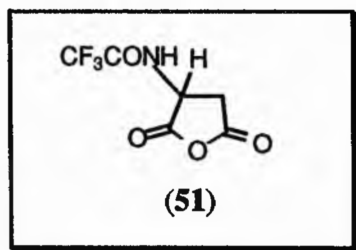


Reagents and conditions: (i) Ac_2O; then AcOH/HBr; (ii) CH_3OH

Scheme 2.1: *Kovacs's synthesis of α–methyl aspartic acid.*

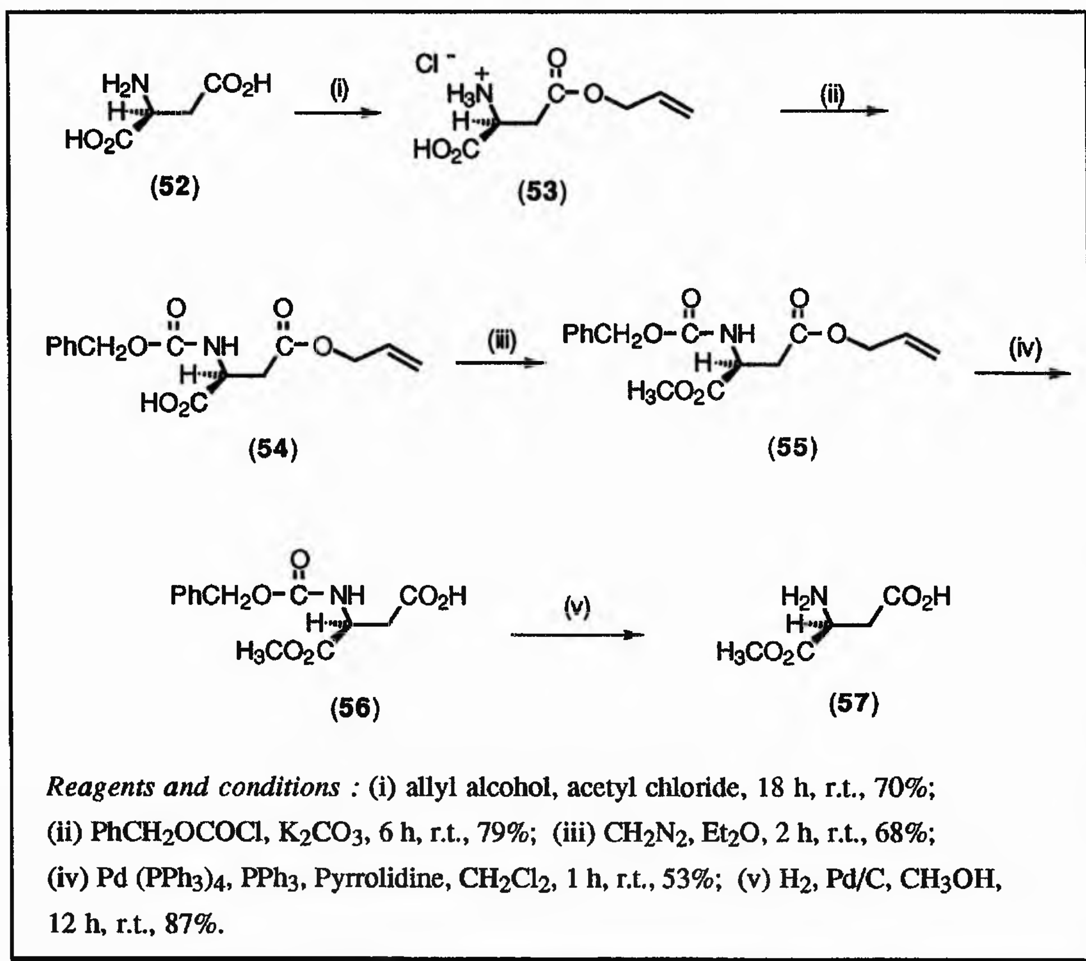
In the absence of the inductive effect, the nucleophilic attack is nearly equally distributed between the α -and β- carboxyl groups as in the polymerization of the free aspartic anhydride to α,β-poly-L-aspartic acid.[119] Aspartic anhydride hydrobromide (**49**) was prepared directly from carbobenzoxy-(2*S*) or (2*R*) aspartic acid by successive treatment with acetic anhydride and dry hydrogen bromide. Gani *et al.*[121] reported the formation of α-methyl ester *via* ring opening of the cyclic trifluoroacetyl aspartic anhydride (**51**) with methanol as 80% of the total mixture. In view of this low selectivity and difficulty encountered with the purification, we decided to

develop a new synthetic route for the preparation of the α-methyl ester of (2*S*)-aspartic acid .



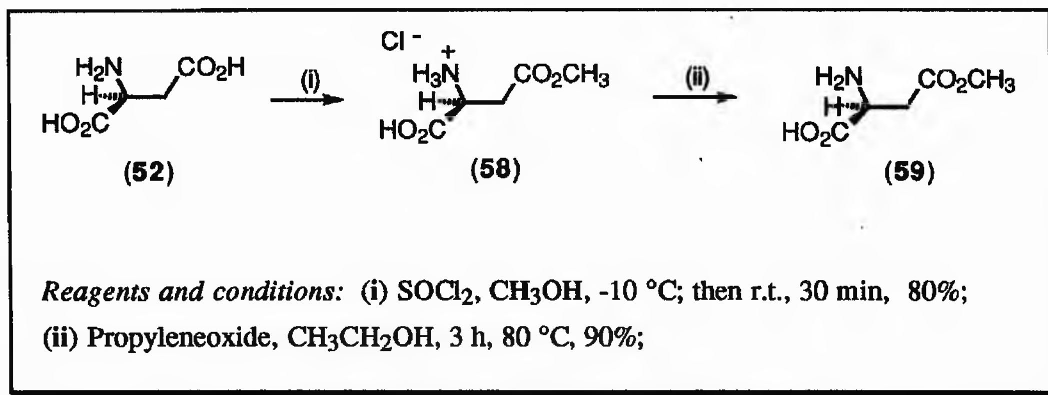
(51)

Following the method of Baldwin *et al.*,[122] (2*S*)-aspartic acid **(52)** was reacted with allyl alcohol in the presence of acetyl chloride to give β-allyl aspartic acid ester hydrochloride salt **(53)** in 70% yield (Scheme 2.2). Benzyl carbamate protection of the α-amino group followed by esterification using ethereal diazomethane gave the fully protected aspartate **(55)** in 68% yield. Deprotection of the allyl group with palladium (Pd^0) followed by catalytic hydrogenolysis of the benzyloxy group gave the required α-methyl aspartate ester **(57)** in 87% yield, m.p. 169-71 °C (lit.,[119] 167 °C); $[\alpha]_D$ 38.40 (*c* 0.5, H_2O) {lit.,[119] $[\alpha]_D^{28}$ +37.60 (*c* 0.5, H_2O)} .



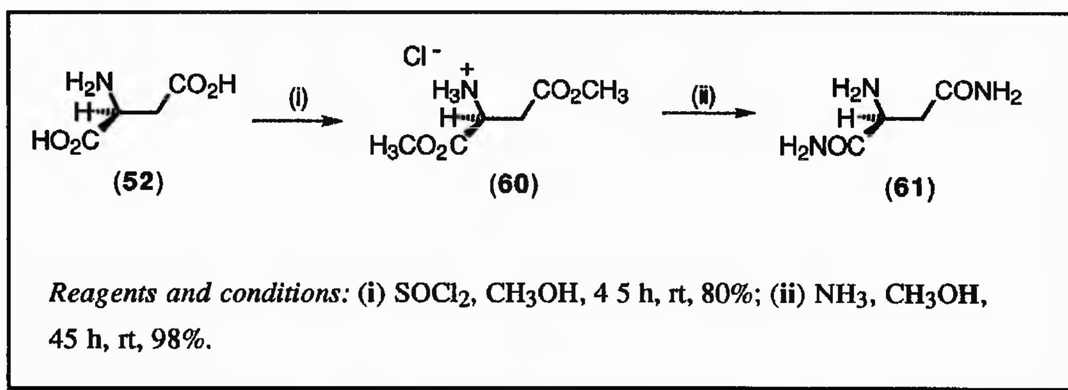
Scheme 2.2: *Synthesis of the α-methyl ester of aspartic acid.*

(2*S*)-Aspartic acid β-methyl ester hydrochloride (**58**) was prepared from (2*S*)-aspartic acid (**52**) and methanol following the method of Goodman and Boardman[123] in 80% yield. The m.p. of (**58**) was 189-90 °C, which was quite different from that quoted by Goodman and Boardman (m.p. 204 °C). However, Schwarz, *et al.*,[124] had reported a m.p. of 191-193 °C, which was in close agreement to our value. Refluxing the hydrochloride salt with propylene oxide gave the (2*S*)-aspartic acid β-methyl ester (**59**) in 90% yield, m.p. 190 °C (lit.,[125] 194-195 °C), (Scheme 2.3).



Scheme 2.3: *Synthesis of the β–methyl ester of aspartic acid.*

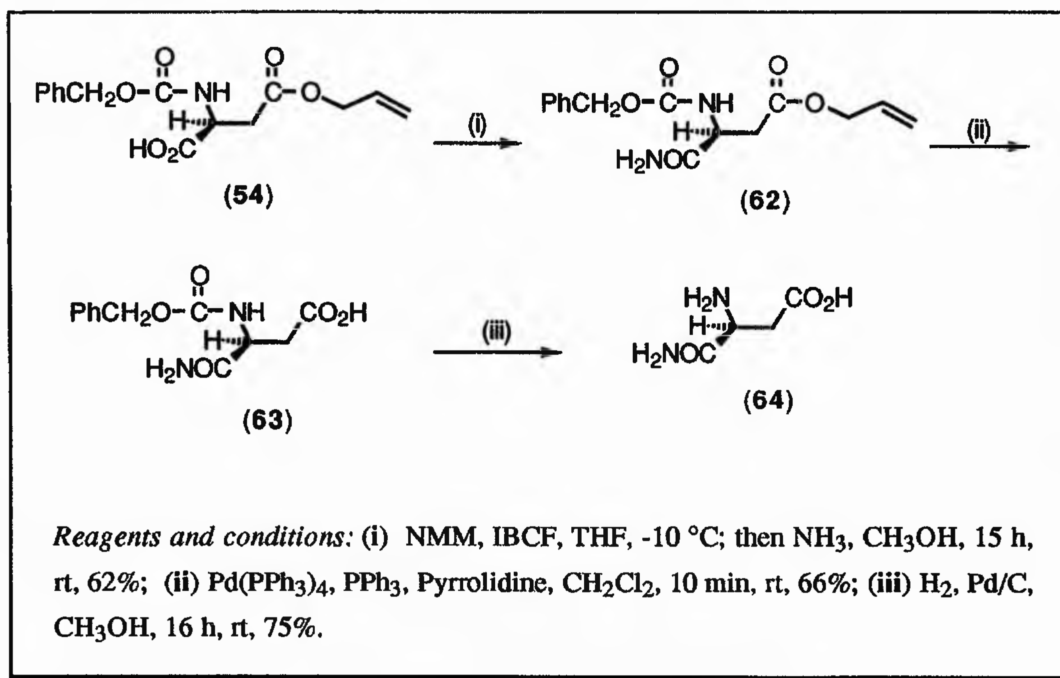
(2*S*)-Aspartic acid α,β-dimethylester (**60**) was prepared by the acid-catalysed esterification of (2*S*)-aspartic acid in methanol acccording to the method of Gmeiner *et al*[125] to give the hydrochloride salt in 80% yield, m.p. 100-101 °C (lit.,[125] 114-15 °C). Treatment of the dimethyl ester with a concentrated solution of ammonia in methanol for 45 h gave the α,β-diamide (**61**) in near quantitative yield, m.p. 214 °C (decomp.); $[\alpha]_D$ +8.25 (*c* 0.4, water), (Scheme 2.4).



Scheme 2.4: *Synthesis of (2S)-Aspartic acid α,β-diamide.*

In order to synthesise the α-monoamide of (2*S*)-aspartic acid it was decided to utilise the β-allyl ester of *N*-benzyloxycarbonyl aspartic acid (**54**) as the starting material. Formation of the isobutyl carbonic mixed anhydride followed by treatment with ammonia gave the protected α-amide (**62**), m.p. 86 °C; *m/z* (Found: M$^+$, 306.1208.

$C_{15}H_{18}N_2O_5$ requires 306.1215), (Scheme 2.5). The use of excess ammonia in this reaction resulted in a side reaction. The α-amide was deprotected by treatment with palladium (Pd^0) and subsequent catalytic hydrogenation using Pd/C effected the removal of the allyl and the benzyl groups, respectively, to yield the α-monoamide (**64**) in 75% yield, m.p. 214-216 °C (decomp.) {lit.,[118] m.p. 212-214 °C (decomp.)}; $[\alpha]_D$ +16.7 (*c* 1.8, 0.1 mol dm^{-3} HCl) {lit.,[118] $[\alpha]_D^{26}$ +15.4 (*c* 1.8, 0.1 mol dm^{-3} HCl)}.



Reagents and conditions: (i) NMM, IBCF, THF, -10 °C; then NH_3, CH_3OH, 15 h, rt, 62%; (ii) $Pd(PPh_3)_4$, PPh_3, Pyrrolidine, CH_2Cl_2, 10 min, rt, 66%; (iii) H_2, Pd/C, CH_3OH, 16 h, rt, 75%.

Scheme 2.5: *Synthesis of isoasparagine.*

The pH dependence of the ^{13}C chemical shift of the α- and β-carbons of aspartate and some of its analogues substituted at the α- and/or β-carboxylate were monitored to determine experimentally pKa changes expected as a result of neutralization of the carboxylates. Table 2.1* provides the summary of the analogues for which pKa's were measured, with aspartate serving as a reference.

* These pKa values were determined by Profs. Klaus Schnackerz and Paul Cook of the Deparment of Biochemistry and Molecular Biology, University of North Texas Health Science Center.

Table 2.1: *Experimentally Determined α-Amine pKa values[a] and Calculated Proton Affinities[b] for Aspartate Analogues*

Compound	pKa	Proton Affinity (KJ/mol)
(2*S*)-Aspartate α,βcarboxylates unprotonated	9.82 (a), 9.84 (b)	-1657.53
β-carboxylate unprotonated	ND	1238.25
α-carboxylate unprotonated	ND	-1284.28
α,β-carboxylates protonated	ND	-870.77
Methylesters		
α-monomethylester	7.84 (a), 7.4 (b)	-1251.31
β-monomethylester	8.72 (a), 8.73 (b)	-1295.28
α,β-dimethylester	6.70 (a), 6.70 (b)	-897.13
Carboxyl amides		
α-monoamide	7.97 (a), 7.99 (b)	-1255.49
β-monoamide	9.07 (a), 9.04 (b)	-1298.63
α,β–diamide	7.02 (a), 6.99 (b)	-927.59
Carboxyl-guanidinium H-bonds[c]		
α-guanidinium complex	ND	1319.25
β-guanidinium complex	ND	1319.63
α,β-bisguanidinium complex	ND	1145.37
Carboxyl-guanidinium H bonds[c]		
α-guanidinium complex	ND	-1392.18
β-guanidinium complex	ND	1394.53
α,β-bisguanidinium complex	ND	-1144.95

[a]pKa values are estimated from the pH dependence of the ^{13}C chemical shift of the α- and β-carbons of (2*S*)-aspartate and its analogs.

[b]Proton affinities were calculated as stated in the text.

[c]Proton affinities were calculated for a system in which two hydrogen-bonds are formed between guanidinium and the carboxylate of interest, with either free optimization or a constrained planar complex (see K. Khayer *et. al.*, *Bioorg. Med. Chem. Lett.*, 1996, **6**, in the press).

Modification of the α-carboxylate either by esterification or amidation results in a decrease in the α-amine pKa by about 1.8-2 pH units, modification of the β-carboxylate results in about a 0.8-1 pH unit decreases in the pKa, while modification of both carboxylates is additive, giving a decrease of 2.8-3 pH units. Calculated proton affinities for the above analogues show changes that roughly parallel the changes in pKa values. Whether neutralization of the α-carboxyl is by protonation, esterification or amidation, a decrease in the free energy by 400-418 kJ/mol is observed, while in the case where both are neutralized, the change is 741-787 kJ/mol, suggesting that a change of 200-250 kJ/mol should be obtained for each pH unit change in the pKa of the α-amine. Estimated changes of 360-375 kJ/mol for neutralization of the β-carboxyl are higher than the expected 200-250 kJ/mol, and this likely reflects restricting the β-carboxyl from hydrogen-bonding with the α-amine.

Comparing the Michaelis complex of aspartate aminotransferase with aspartate, complexes in which guanidinium is hydrogen-bonded with the carboxylates were also modeled with free optimization and with a constrained geometry with the latter likely more accurately reflecting the Michaelis complex. The changes in the case of the guanidinium complexes are much smaller than those observed for any of the aspartate analogues or for aspartate protonated at its carboxylates. The smaller changes suggest less than complete charge neutralization. Even with both carboxylates complexed with guanidinium, data suggest a change of >2 but <3 pH units in the pKa of the α-amine. Figure 1* shows the (2*S*)-aspartate Michaelis complex model used with the α-amine of the amino acid either protonated or unprotonated. Hydrogen-bonding distances from the α-carboxylate to the guanidinium of Arg-386 are 2.77 Å and 2.93 Å, while those from the β-carboxyl to Arg-292 are 2.81Å and 3.29 Å. The distances

*Molecular modelling carried out by Dr. J. Wilkie in the School of Chemistry, University of St. Andrews.

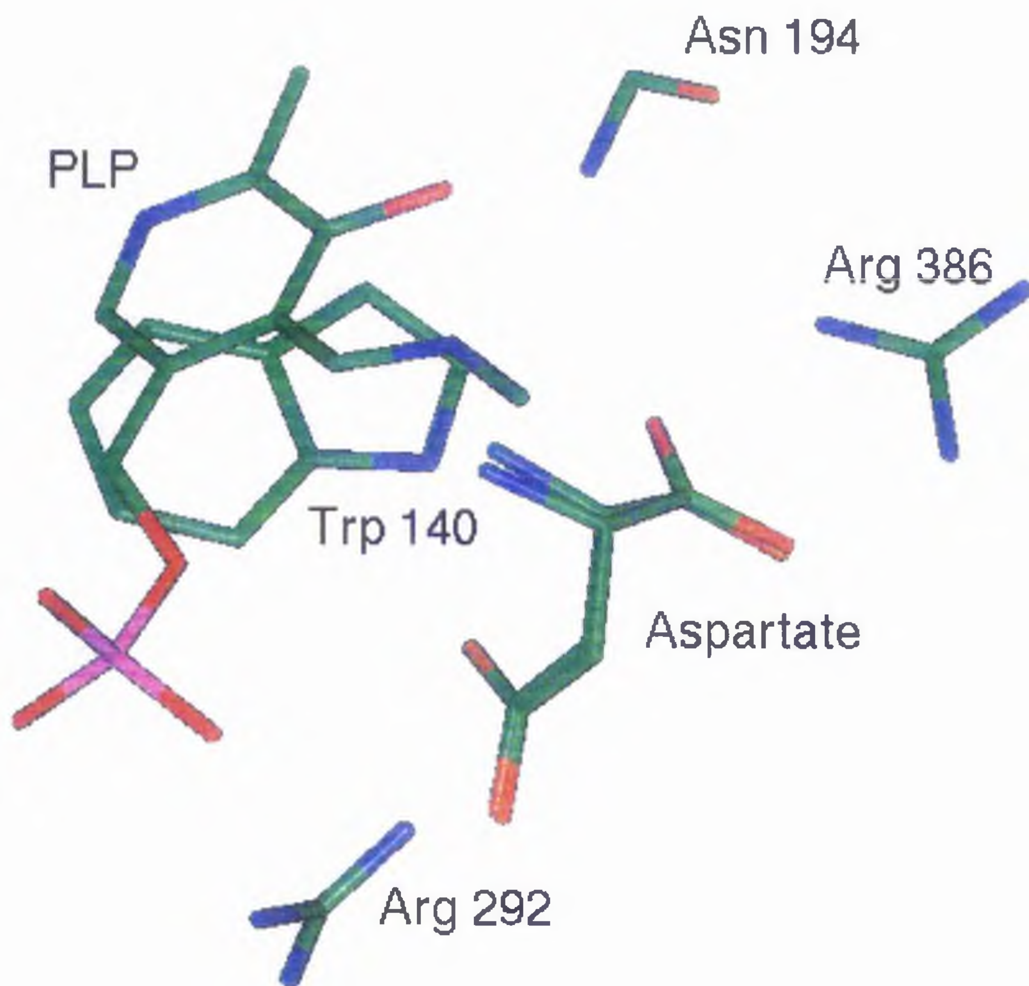


Figure 2.1. *Model of the L-Aspartate Michaelis Complex of Aspartate Aminotransferase. The superposition of two complexes is shown in which the α-amine of L-aspartate is protonated (above) or unprotonated (below). The guanidinium of arginine 386 is shown at the top of the figure, while that of arginine 292 is at the bottom.*

change slightly upon deprotonation of the α-amine, for the α-carboxyl/Arg-386 to 2.77 Å and 2.70 Å, and for the β-carboxyl/Arg-292 to 2.75 Å and 3.27 Å, respectively.

The proton affinity of the α-amine of aspartate in Michaelis complex with aspartate aminotransferase, is calculated as -1320.84 kJ/mol. The difference of 334.72 kJ/mol between the latter and the proton affinity of aspartate with both carboxylates ionized suggests a decrease in the pKa of the α-amine of aspartate by >1 and <2 pH units upon binding of aspartate to the pyridoxamine form of aspartate aminotransferase.[31] Thus, although both of the carboxylates of aspartate form hydrogen-bonds to Arg-292 and Arg-386 upon binding to enzyme, charge is not completely neutralized at either of the two.

α-Substituted β-Carboxylates. The geometry of L-aspartate is such that the β-carboxylate is well placed to accept a hydrogen-bond from the protonated α-amine. Neutralization of the β-carboxylate (by a counter-ion such as guanidinium, or by esterification, etc.) abolishes, or severely curtails, hydrogen-bonding tendency, while in the case of fully ionised aspartate, the zwitterionic species is stable. However, in the case of neutralization of the α-carboxylate alone, the proton affinity of the amine is lowered, relative to the β-carboxylate, sufficiently to abolish the zwitterionic minimum, and this results in the spontaneous transfer of a proton from the amine to the β-carboxylate during the course of optimisation. To determine the effect of neutralization of the α-carboxylate alone on amine proton affinity, amine N-H bonds were fixed at 1.028 Å in the protonated form. The Michaelis Complex of aspartate aminotransferase with L-aspartate was derived from the external aldimine of aspartate aminotransferase with aspartate[126] as obtained from the Brookhaven protein data base as follows.

(1) The correct bonding for the Michaelis complex was established, *i.e*. Lys-258 was reattached *via* Schiff base linkage to the active site PLP, but the external aldimine coordinates were retained.

(2) MM (AMBER) optimisation of the whole protein was carried out to obtain the correct co-ordinates for the Michaelis complex.

(3) Excess protein was deleted leaving only model system including the bound aspartate, PLP, the two guanidinium groups of arginines 386 and 292 that form a double hydrogen-bond with the carboxylates of aspartate, along with Try-140 and Asn-194 which are in close proximity to the bound aspartate, Figure 2.1.

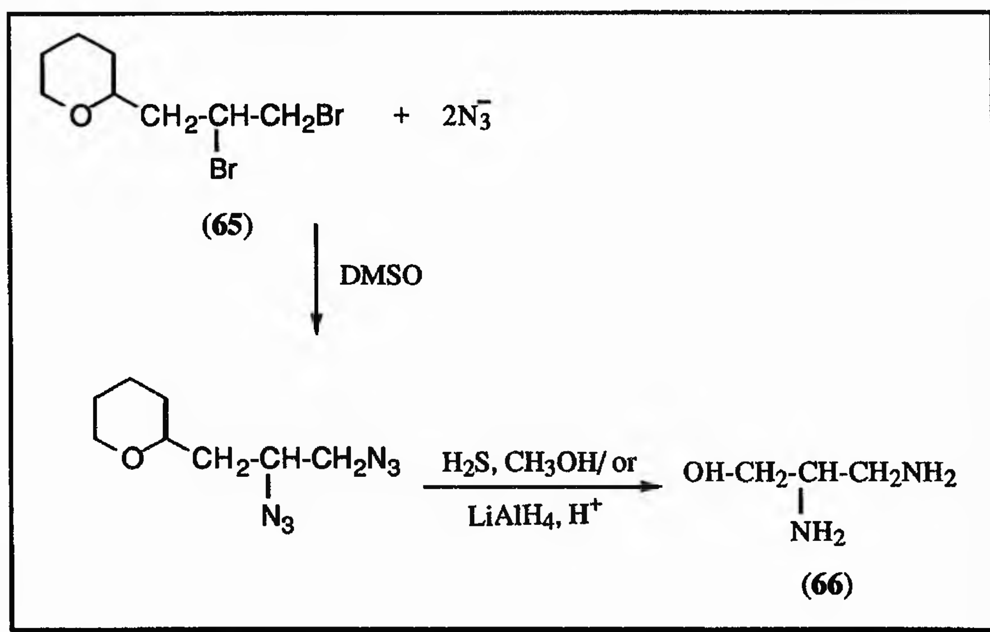
(4) The coordinates of the remaining (those listed in 3 above) protein atoms were fixed, and the position and internal geometry of the bound aspartate was optimised.

2.2. Synthesis of 2,3-diaminopropan-1-ol hydrogensulfate ester

Glutamate 1-semialdehyde aminomutase, as mentioned earlier (Chapter 1, p 16) is an exceptional enzyme in that, although it is classified as a mutase, it actually functions as an aminotransferase (transferring amino and oxo functions within the molecule).

In order to shed more light on the mechanism of this enzyme, it is important for us to design and synthesis substrates and inhibitors. One such substrate/inbibitor would be 2,3-diaminopropan-1-ol hydrogen sulfate ester (**74**). Although this compound has not been reported in the literature, it's possible precursor, 2,3-diamino-1-propanol[127] has attracted more attention due to its involvement in antitumor activity. Platinum (II) complexes of 2,3-diamino propanol (pnOH) isomers shows antitumor activities against leukemia L1210.[128] Among the Pt (II) complexes containing pnOH isomers, R-pnOH showed higher antitumor activity than those containing S- or racemic pnOH.

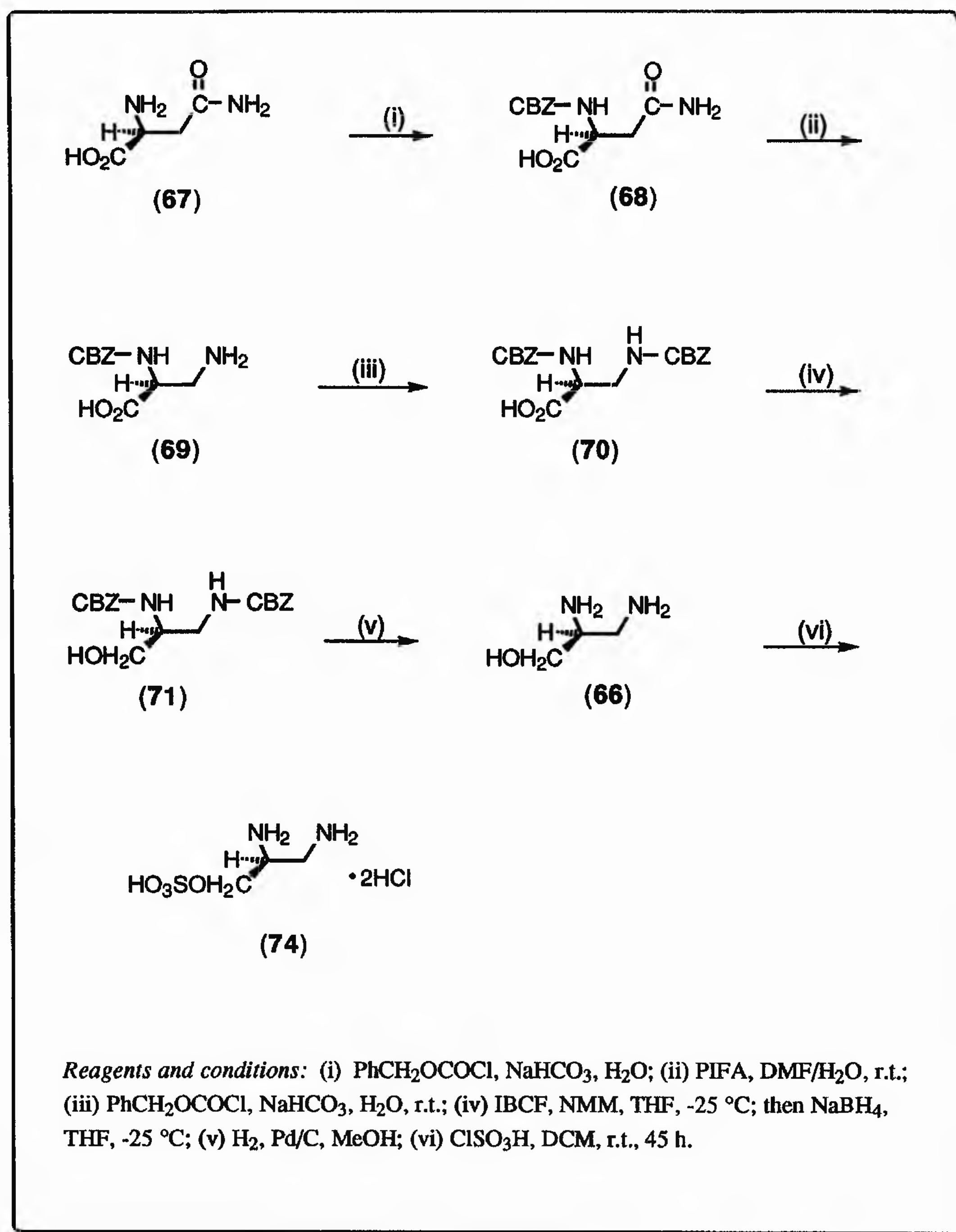
There are very few syntheses in the literature for the preparation of 2,3-diaminopropan-1-ol. Okamoto *et al.*[127] have prepared 2,3-diaminopropan-1-ol (**66**) by reaction of the tetrahydropyran derivative of 2,3-dibromopropan-1-ol (**65**) with sodium azide in DMSO followed by reduction with $LiAlH_4$ in THF or with H_2S in methanol (Scheme 2.6). Resolution of the racemic 2,3-diaminopropanol was achieved by a modification of the method used in the case of 1,2-propane diamine.[128] The dihydrochloride of 2,3-diaminopropanol was then converted into the free form by passing the salt through a column of AmberliteIRA-410 resin.



Scheme 2.6: *Preparation of 2,3-diaminopropan-1-ol .*

Considering the potent antitumor activity and the need to unravel the mechanism of glutamate 1-semialdehyde it was important for us to develop a new synthetic route to 2,3-diaminopropan-1-ol.

Benzyloxycarbonyl protection of (2*S*)-asparagine (**67**) using Schotten-Baumann conditions gave (**68**) in 78% yield, m.p. 160-62 °C (decomp.) {lit.,[137] 165 °C (decomp.)}; $[\alpha]_D$ +6.4 (*c* 1.6, AcOH) {lit.,[137] $[\alpha]_D$ +7.8 (*c* 1.6, AcOH)}, (Scheme 2.7).



Reagents and conditions: (i) $PhCH_2OCOCl$, $NaHCO_3$, H_2O; (ii) PIFA, DMF/H_2O, r.t.; (iii) $PhCH_2OCOCl$, $NaHCO_3$, H_2O, r.t.; (iv) IBCF, NMM, THF, -25 °C; then $NaBH_4$, THF, -25 °C; (v) H_2, Pd/C, MeOH; (vi) $ClSO_3H$, DCM, r.t., 45 h.

Scheme 2.7: *Synthesis of 2,3-diaminopropyl hydrogensulfate.*

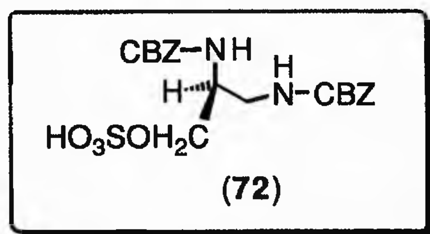
The amide was then converted into the amine (**69**) (m.p. 230-232 °C {lit.,[129] m.p. 228-230 °C (decomp.)}; $[\alpha]_D$ -6.9 (*c* 0.4, 1 mol dm^{-3} NaOH) {lit.,[129] $[\alpha]_D$ -7.8 (*c* 0.4, 1 mol dm^{-3} NaOH)}) using the conditions of Waki,[129] phenyl iodosyl *bis* (trifluoroacetate) (PIFA) in a mixture of DMF and water. Subsequent protection of

the free amino group using again benzylchloroformate gave the *bis*-Cbz protected diamine (**70**) in 92% yield. Reduction of **70**, was first tried with borane (3 equivalent) in THF, however even after 16 h reflux the peaks at δ 8.17 and δ 173.84 in the ^{1}H- and ^{13}C-NMR spectrum were still present. Using 5 equivalences of borane and refluxing the mixture for 4 h showed the appearance of a new spot on the TLC plate.

The ^{1}H-NMR spectrum of the crude mixture was quite complex and it was difficult to access whether or not some reduction had occured. However the ^{13}C-NMR spectrum was more promising and showed an additional peak for the alcoholic CH_2 at δ 62.1 ppm. Refluxing the reaction for 2 days did improve the ratio of product to starting material. However, due to the problems with the extraction and purification of this product, we decided to pursue alternative methods. Reduction using $LiAlH_4$ (1.2 equivalent) at 0 °C was also unsuccessful probably due to the formation of the lithium salt of the acid. In order to overcome this problem, the methyl ester (**75**) of (**70**) was prepared and subjected to the same reduction conditions. Although the ^{13}C-NMR was again encouraging, problems with purification (by column chromatography) and extraction led us to abandon this strategy. The α-acid was however, successfully transformed into alcohol (**71**) by reduction of the preformed *bis*($N^{2,3}$-benzyloxycarbonyl)-propan isobutylcarbonic anhydride with $NaBH_4$ at -30 °C, but in a moderate 68% yield. ^{13}C-NMR spectrum showed the complete disappearance of the acid carbonyl peak (at δ 173.84) and an additional peak at δ 62.1 corresponding to the alcohol (**71**) {m.p. 96-97 °C; *m*/*z* (Found: $[M + H]^+$, 373.1387. $C_{19}H_{21}N_2O_6$ requires 373.1399)}.

Sulfation of the diamino protected alcohol (**71**) with chlorosulfonic acid in trifluoroacetic acid following the method of Previero[130] was unsuccessful. Although, reaction with chlorosulfonic acid in DCM gave a white solid of m.p. 145-150°C (slow), the mass spectrum showed the compound had a molecular weight of 486,

which was not consistent with the desired product **(72)**. Due to its insolubility and rapid decomposition we were unable to characterise this compound.

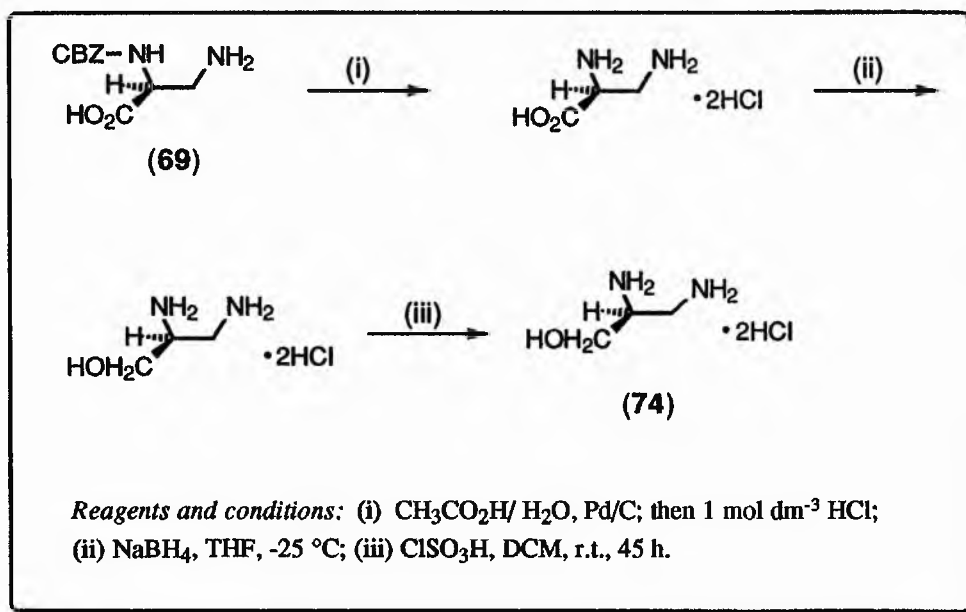


In an attempt to aid the sulfation reaction we decided to introduce the sulfate after the removal of the amino protecting group from the alcohol. Removal of the benzyloxycarbonyl protecting groups by catalytic hydrogenation using palladium on activated carbon gave the free diamino alcohol **(66)** in 93% yield. This was then converted into the dihydrochloride salt **(73)** for full characterisation purposes, m.p. 180-81 °C (lit.,[127] 172-74 °C). The ^{13}C-NMR spectrum possessed only three peaks, at δ 61.98, 52.87 and 41.36, consistent with the alcoholic CH_2, α-CH and β-CH_2 carbons respectively.

Attempted sulfation of the diamino alcohol **(66)** using the method of Previero[130] was again unsuccessful. However, reaction with chlorosulfonic acid in dichloromethane gave the desired compound **(74)** as a hydrochloride salt. The ^{13}C-NMR spectrum showed the partial disappearance of the alcoholic peak and the presence of a new peak at δ 67.44, indicates the formation the sulfated product. Attempted purification of the crude material by ion-exchange column chromatography using strong and weak cation exchangers met with failure. However recrystallisation from water/ ethanol gave white solid, m.p. 55-60 °C, which was > 80% pure as judged by NMR spectroscopy.

2.2.1 Future Work

We have developed a successful route to 2,3-diamino propyl hydrogensulfate starting from (2*S*)-asparagine, although the final compound obtained was not pure spectroscopically.* In order to improve the yield and purity of our target, a possible adaptation of the synthesis of Waki *et al.*[129] could be considered. They have sucessfully converted N^2-benzyloxycarbonyl-(2*S*)-2,3-diaminopropanoic acid into (2*S*)-2,3-diamino propanoic acid hydrocloride in 93% yield. Reduction of this propanoic acid with $NaBH_4$ and subsequent sulfation of that amino alcohol with chlorosulfonic acid would give the desired sulfated product (Scheme 2.8).



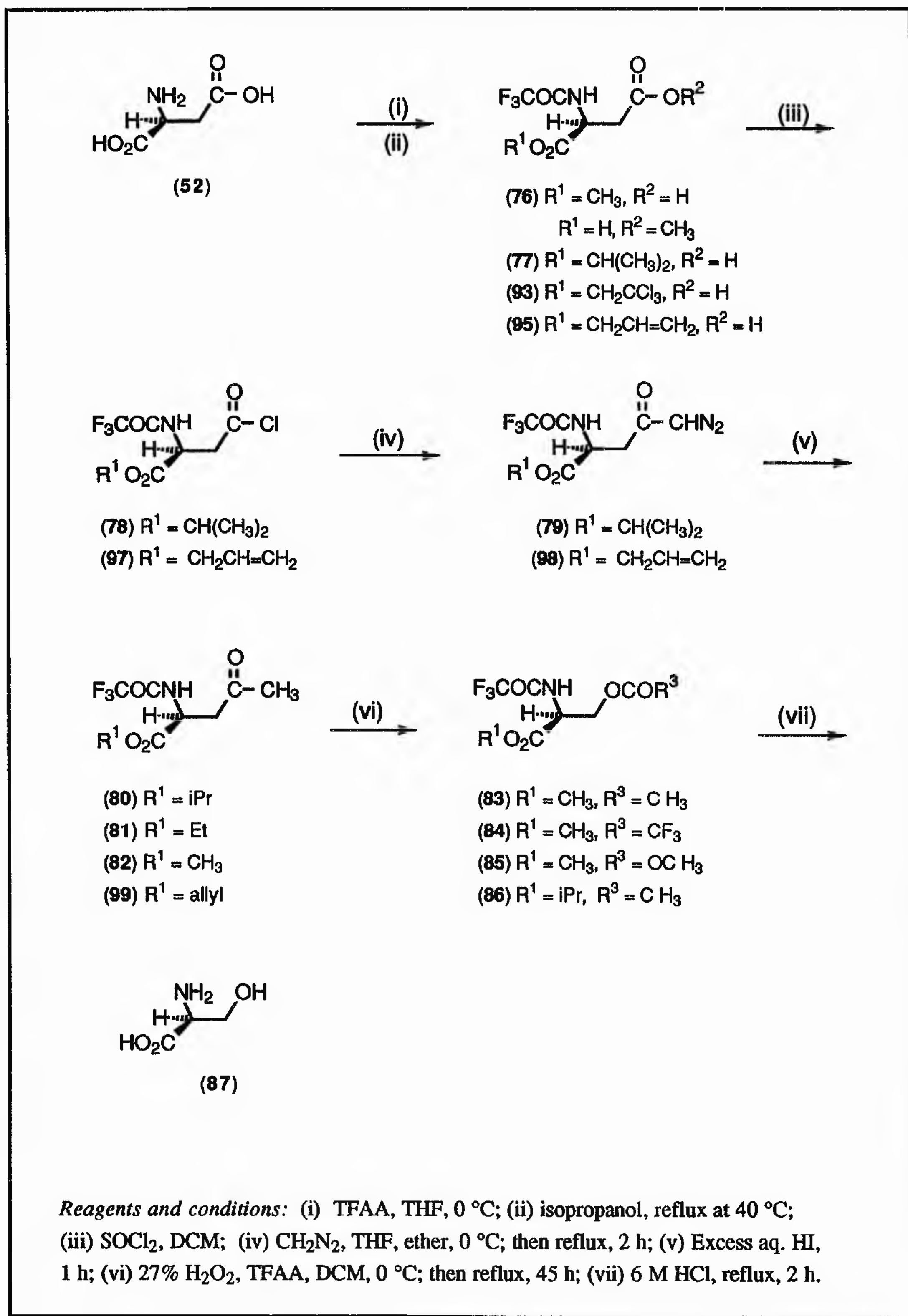
Scheme 2.8 : *A possible synthesis of 2,3-diamino propyl hydrogen sulfate.*

*This compound has purified by ion-exchange chromatography and was used in kinetic studies in the laboratory of Prof. Robert John.

2.3 Synthesis of 3-[^{2}H]-serine

The amino acid (2*S*)-serine is involved in many biological reactions in which substitution or elimination occurs at C-3.[131] The availability of (2*S*)-serine, chirally labelled at C-3 with isotopic hydrogen would allow the stereochemical course of some of these reactions to be assessed. Although several synthesis of (2*S*)-serine stereospecifically labelled at C-3 with tritium[132] or deuterium[121,132c,133] have been reported in the literature, they are either very low yielding or the amount of incorporation of ^{3}H or ^{2}H is insufficient to carry out any meaningful studies. Gani and Young, however have successfully synthesised C-3 chirally deuterated-(2*S*)-serine in 8.5% yield with >95% incorporation of deuterium.[121] The key reaction in their sequence was a Baeyer-Villiger rearrangement at a primary migrating centre. We decided to follow this protocol in a hope of improving the yield of the Baeyer-Villiger reaction (lowest yielding step in the synthesis, 20%) so as to obtain a better overall yield.

The synthesis of unlabelled serine was first pursued, in order to investigate the rearrangement further. (2*S*)-Aspartic acid (**52**) was treated with trifluoroacetic anhydride and the intermediate anhydride was treated with methanol *in situ,* following the method of Weygand.[134] The ^{1}H-NMR spectrum of the crude product showed that two methyl esters , (**76**), (R^1 = Me, R^2 = H) and (R^1= H, R^2 = Me) were present in the ratio 4:1 as judged by integration of the methoxy singlets at 3.79 and 3.71 ppm respectively (Scheme 2.9).



Reagents and conditions: (i) TFAA, THF, 0 °C; (ii) isopropanol, reflux at 40 °C; (iii) $SOCl_2$, DCM; (iv) CH_2N_2, THF, ether, 0 °C; then reflux, 2 h; (v) Excess aq. HI, 1 h; (vi) 27% H_2O_2, TFAA, DCM, 0 °C; then reflux, 45 h; (vii) 6 M HCl, reflux, 2 h.

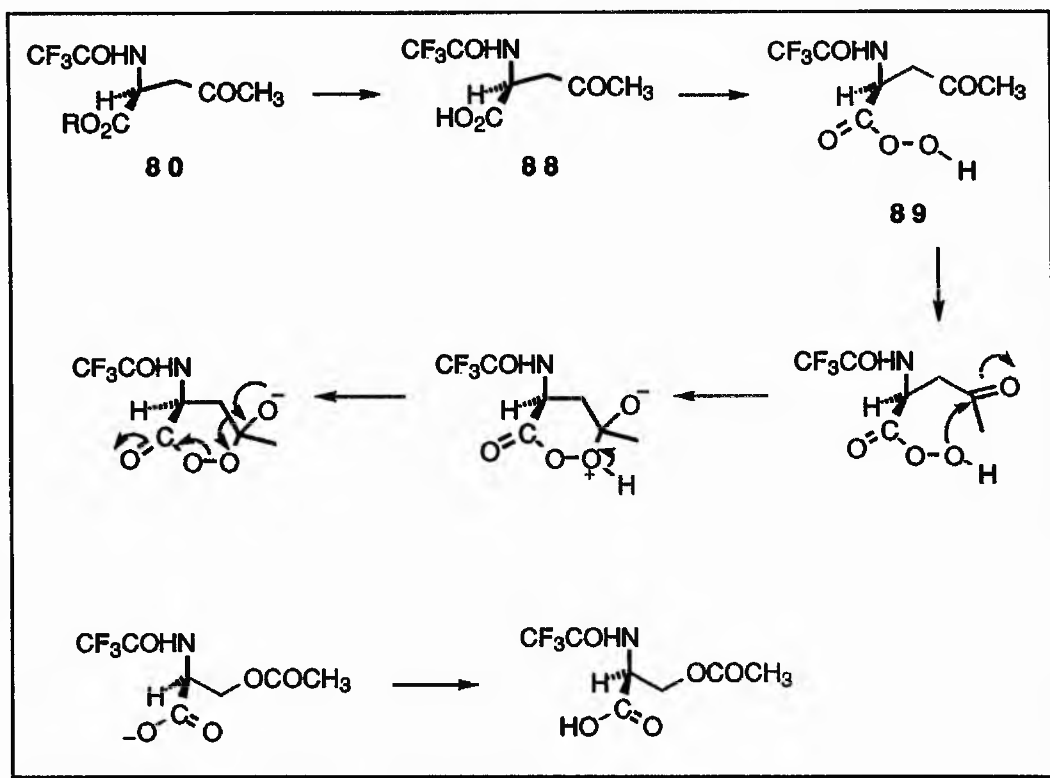
Scheme 2.9: *Synthesis of serine.*

In an attempt to improve this selectivity, we have decided to use isopropanol as the solvent. The reaction was found to be stereospecific since only one set of signals for the isopropyl group were seen in the ^{1}H-NMR spectrum, at 1.27 ppm for the methyl protons and at 5.11 ppm for the isopropyl C-2 proton. Extra bulk of the isopropanol makes it more selective nucleophile and sucesptible to attack at the more reactive α-carbonyl carbon of cyclic trifluoroacetic anhydride (**51**). The α–isopropyl-*N*-trifluoroacetyl-β-aspartic acid ester (**77**) was then refluxed with excess thionyl chloride. This was found not to be a reliable method for the formation of the acid chloride, due to the reformation of cyclic anhydride. However, refluxing in dichloromethane using 4 equivalents of thionyl chloride gave the required acid chloride (**78**) in 75% yield, m.p. 120-123 °C (lit.,[138] 123-5 °C); $[\alpha]_D$ +65.4 (*c* 0.63, CH_2Cl_2) {lit.,[138] $[\alpha]_D$ +65.8 (*c* 0.63, CH_2Cl_2)}. Reaction with freshly prepared alcohol free and moisture free excess ethereal diazomethane then gave the desired diazoketone (**79**) in 95% yield after recrystallisation from diethyl ether/ cyclohexane. The compound showed an absorbance at 2126 cm^{-1} in the infra-red, and exhibited a singlet at 5.32 in the ^{1}H-NMR spectrum, (m.p. 91-93 °C; *m/z* (Found: $[M + H]^+$ 296.0856. $C_{10}H_{13}N_3O_4F_3$ requires 296.0858). Treatment of the diazoketone with an excess of aqueous hydriodic acid in methylene chloride then gave the methyl ketone (**80**) in 78% yield, (Found: C, 44.45; H, 5.3; N, 5.22. $C_{10}H_{15}NO_4F_3$ requires C, 44.6; H, 5.25; N, 5.2%). The ^{1}H-NMR spectrum showed a methyl ketone signal at 2.18 ppm and the disappearence of the diazoketone signal at 5.32 ppm.

Having obtained the desired α-isopropyl methyl ketone (**80**), we were unable to induce this to undergo Baeyer-Villiger oxidation under a wide variety of conditions. As 90% hydrogen peroxide, for making trifluroroperoxyacetic acid (the grade used by Gani and Young) was also no longer commercially availability, we used three times as much 27% H_2O_2 for making trifluoroperoxyacetic acid. The Baeyer-Villiger oxidation was attempted using different reaction times, temperature, solvent and neat (without solvent), and in each case no peaks corresponding to the product in the

^1H-NMR spectrum could be observed. Even when the oxidation was carried out using alternative peracids; peroxyacetic acid and peroxymalic acid (from 27% H_2O_2) no reaction occured. It, therefore appears, that although the isopropyl group enhances regioselectivity in the opening of the cyclic anhydride (**51**), the Baeyer-Villiger reaction under a variety of conditions is unsuccessful for the ethyl (**81,** R^1=Et) and isopropyl esters (**80**, R^1=$CH(CH_3)_2$). This is quite different from the results reported by Gani and Young, who, in the case of the methyl ester (**82,** R^1=CH_3) observed three compounds; the desired acetate (**83,** R^1=CH_3, R^2=CH_3), the transesterification product (**84**, R^1=CH_3, R^2=CF_3) and the diester (**85**, R^1=CH_3, R^2=OCH_3) as a result of methyl migration which was an unexpecting result.[121]

The failure of this Baeyer-Villiger oxidation leads us to pursue an alternative route to the desired acetate (**86**). We decided to try and make use of the α-peracid (**89**) in a hope that we may get intramolecular ring closure to form the Criegee intermediate, with subsequently breakdown of the cyclic intermediate to give the desired acetate ester (Scheme 2.10).



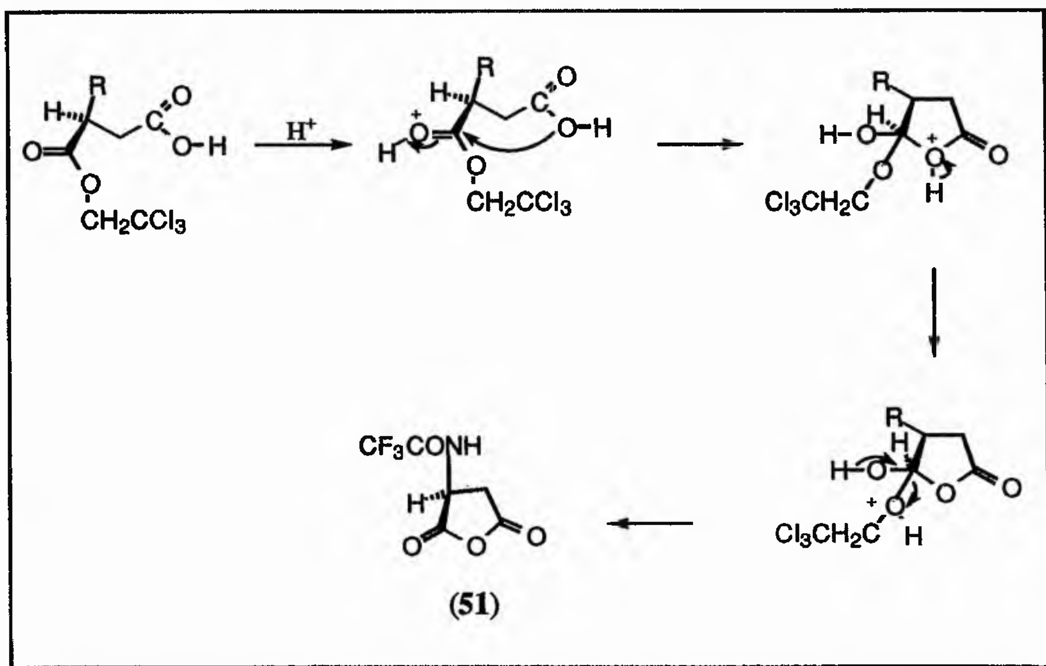
Scheme 2.10: *An alternate method for the preparation of acetate by Baeyer Villiger oxidation.*

The synthesis of the free α-acid by simple hydrolysis of the ester **(80)** proved more difficult than expected, as the trifluroacetyl group was very base labile. Attempted hydrolysis of α-isopropyl methyl ketone with NaOH/CH_3OH led to hydrolysis of both the trifluoroacetyl and isopropyl groups **(92)**. Disappearance of the isopropyl peaks at δ 1.23 & 5.05 and at δ 21.49 and 70.43 in the ^{1}H-NMR and ^{13}C-NMR spectrum respectively indicated removal of isopropyl group. A new peak at δ 173.45 in the latter spectrum was also indicative of the presence of a free carboxylic acid. The TLC of this reaction product also showed a positive ninhydrin test.

Since we were unable to hydrolyse the α-isopropyl group without affecting the trifluoroacetyl group, we decided to replace the α-ester with a more labile group, that could be removed in the presence of the trifluoroacetyl group.

In the literature, the 2,2,2-trichloroethyl group has been found to be very labile upon treatment with Zn/acetic acid.[135] We reasoned that the trifluoroacetyl group would be unaffected by these condition. Treatment of the cyclic anhydride (**51**) with neat trichloroethanol at r.t. for 45 h gave the trichloroethyl ester (**93**) in only 50% yield. ^{1}H-NMR spectrum of the crude product in C^2HCl_3 showed peaks at δ 4.82 (m) and δ 8.17 (br) indicating the presence of $-OCH_2CCl_3$ and $-CO_2H$ group (exchangeable with D_2O). Addition of ethereal diazomethane to this reaction mixture resulted in two methoxy singlet at δ 3.71 and δ 3.80 (the ratio of α- and β-methyl ester is 1:2) which indicating that the opening of the cyclic anhydride with trichloroethanol was not regioselective.

Refluxing the cyclic anhydride with 3 equivalents of trichloroethanol in THF under N_2 for 7 days caused extensive decomposition. An improved yield (90% before purification) was obtained when the cyclic anhydride was treated with excess trichloroethanol and then stirred vigorously at 40 °C under N_2 overnight. But when the α-trichloroethyl ester-β-acid was subjected to reaction with thionyl chloride no reaction occurred. The 300 MHz ^{1}H-NMR spectrum showed no CO_2H peak, however, additional peaks at δ 3.04-3.38 (2 H, m, β-CH_2), δ 4.98 (1 H, m, α-CH) and δ 10.23 (1H, d, NH) indicating the reformation of cyclic anhydride (**51**), due to loss of the trichloroethyl group in presence of traces of HCl in the reaction medium (Scheme 2.11).



Scheme 2.11: *Reformation of cyclic anhydride in presence of HCl.*

The allyl ester seemed like a possible alternative, as it can be removed under very mild conditions using Palladium (Pd^0).

Reaction of the cyclic anhydride **(51)** with allyl alcohol gave the allyl ester **(95)** in 94% yield, {m.p. 86-88 °C; *m/z* (Found: $[M + H]^+$, 270.0597. $C_9H_{11}NO_5F_3$ requires 270.0589)}. Treatment of the ester with diazomethane showed two methoxy singlet at δ 3.71 and δ 3.80 indicating the presence of β- and α- methyl ester in a ratio of 5:1. Conversion of the α-allyl ester-β-acid **(95)** to α-allyl ester-β-methyl ketone **(99)** was carried out as previously described above (Scheme 2.9). Initial attempts at the deprotection of the α-allyl ester using palladium (Pd^0) in presence of triphenyl phosphine and pyrrolidine has been unsuccessful.

2.3.1 Future Work

In the protocol for the synthesis of serine described above, the Baeyer-Villiger was the key reaction and the most challenging step (Scheme 2.9). Various attempts to optimise this oxidation have so far been unsuccessful. One possible alternative which could be tried would be to prepare the α-*p*-methoxy benzyl ester. This group could be deprotected in presence of trifluroacetyl group by oxidative cleavage under essentially neutral conditions using DDQ in CH_2Cl_2.

Once optimised conditions for the Baeyer-Villiger oxidation are found, the synthesis of serine deuterated at C-3 starting would essentially start from C-3 [^{2}H]-aspartic acid using the same chemistry. Aspartic acid, labelled in the C-2, or either of the C-3 positions can be readily obtained from fumaric acid.[121]

Chapter Three

EXPERIMENTAL

3.0 Experimental

Elemental microanalyses were performed in the departmental microanalytical laboratory.

NMR spectra were recorded on a Varian-300 (300 MHz; f.t. ^{1}H-NMR, and 74.46 MHz; ^{13}C-NMR), or a Varian gemini 200 (200 MHz; f.t. ^{1}H-NMR and 50.31 MHz; ^{13}C-NMR) spectrometers. ^{1}H-NMR spectra are described in parts per million downfield shift from TMS and are reported consecutively as position (δ_H or δ_C), relative integral, multiplicity (s -singlet, d -doublet, t -triplet, q -quartet, d d -double of doublets, sep -septet, m -multiplet, and br -broad), coupling constant (Hz) and assignment (numbering according to the IUPAC nomenclature for the compound). ^{1}H-NMR were referenced internally on ^{2}HOH (4.68 ppm), $CHCl_3$ (7.27 ppm) or DMSO (2.47 ppm). ^{13}C-NMR were referenced on CH_3OH (49.9 ppm), C^2HCl_3 (77.5 ppm), or DMSO (39.70 ppm). IR Spectra were recorded on a Perkin-Elmer 1710 f.t. IR spectrometer. The samples were prepared as Nujol mulls, solution in dichloromethane or thin films between sodium chloride discs. The frequencies (υ) as absorptions maxima are given in wavenumbers (cm^{-1}) relative to a polystyrene standard.

Mass spectra and accurate mass measurements were recorded on a VG 70-250 SE or a Kratos MS-50 spectrometers. Fast atom bombardment spectra were recorded using glycerol as a matrix. Major fragments were given as percentages of the base peak intensity (100%).

Flash chromatography was performed according to the method of Still *et al.*[136] using Sorbsil C 60 (40-60 µm mesh) silica gel. Analytical thin layer chromatography was carried out on 0.25 mm precoated silica gel plates (Macherey-Nagel SIL g/UV254) and compounds were visualised using UV fluorescence, iodine vapour, ethanolic phosphomolybdic acid, or ninhydrin.

Melting points were taken on an Electrothermal melting point apparatus and are uncorrected. Optical measurements were measured at 25 °C on a Optical Activity AA-1000 polarimeter using 1 dm path length cells; $[\alpha]_D$ values are given in deg cm^2 g^{-1}.

The solvents used were either distilled or of analar quality and light petrol ether refers to that portion boiling between 40 and 60 °C. Solvents were dried according to literature procedures. ethanol and methanol were dried using magnesium turnings. Isopropanol, CH_2Cl_2 and pyridine were distilled over CaH_2. THF and diethyl ether were dried over sodium/benzophenone and distilled under nitrogen. Thionyl chloride was distilled over sulfur, and the initial fractions were always discarded.

β-Allyl-(2*S*)-aspartate hydrochloride (53)

Acetyl chloride (28 cm^3, 39 mmol) was added dropwise to freshly distilled allyl alcohol (250 cm^3) at 0 °C. The solution was stirred for 10 min at 0 °C and then for a further 1 h at room temperature. (2*S*)-Aspartic acid (13.3 g, 100 mmol) was added and the resulting suspension was then stirred overnight. The solution was poured into diethyl ether (150 cm^3) and the resulting white precipitate was filtered to give the β-allyl ester (14.65 g, 70%), m.p. 188-89 °C (lit.,[122] 192 °C); υ_{max} (Nujol)/cm^{-1} 1756 (CO, acid) and 1722 (CO, ester); δ_H (200 MHz; ^2H_2O) 3.06 (2 H, m, β-CH_2), 4.3 (1 H, t, α-CH), 4.59 (2 H, m, OCH_2-CH), 5.27 (2 H, m, CH=CH_2) and 5.9 (1 H, m, CH=CH_2); δ_C (50.3 MHz; ^2H_2O) 38.78 (β-CH_2), 52.83 (α-CH), 59.58 (OCH_2-CH), 121.78 (CH=CH_2), 134.18 (CH=CH_2), 173.54 (CO_2CH_2) and 173.87 (CO_2H); *m/z* (CI) 174 (100%, $[M + H]^+$), 128 (2, $[M - CO_2H]^+$) and 75 (12, $[M + H - C_4H_7O]^+$).

β-Allyl N-Z-(2*S*)-aspartate (54)

β-Allyl-(2*S*)-aspartate hydrochloride (**53**) (10.0 g, 47 mmol) was dissolved in water (200 cm^3) and diethyl ether (70 cm^3) was added. Potassium carbonate (10.37 g, 75 mmol) and benzyl chloroformate (10.69 cm^3, 75 mmol) were added and the resulting solution was stirred for 6 h. The layers were separated, and the aqueous layer was washed with diethyl ether (2 x 70 cm^3), and then acidified with 12 mol dm^{-3} hydrochloric acid. The resulting solution was extracted with diethyl ether (3 x 60 cm^3), dried ($MgSO_4$) and concentrated under reduced pressure to give β-allyl *N*-Z-(2*S*)-aspartate as a colourless oil (11.51 g, 79%); $[\alpha]_D$ +15.8 (*c* 1.1, $CHCl_3$) {lit.,[122] +15.3 (*c* 1.1, $CHCl_3$)}; *m/z* (Found: M^+, 307.1063. Calc. for $C_{15}H_{17}NO_6$: 307.1055); υ_{max} neat/cm^{-1} 2995-3335 (br, CO_2H), 1731 (CO, br, acid and ester) and 1600 (C=C); δ_H (200 MHz; C^2HCl_3) 2.9 (1 H, dd, 1 of β-CH_2), 3.10 (1 H, dd, 1 of β-CH_2), 4.55 (2 H, d, OCH_2-CH), 4.67 (1 H, m, α-CH), 5.15 (2 H, s, OCH_2Ph), 5.28 (1 H, m, *CH*=CH_2), 6.45 (1 H, br s, CO_2H) and 7.32 (5 H, s, ArH); δ_C (50.3 MHz; C^2HCl_3) 36.93 (βCH_2), 50.69 (αCH), 66.36 (O*C*H_2-CH), 67.83 (O*C*H_2Ph), 119.27 (CH=*C*H_2), 128.63 (*ortho* ArC), 128.74 (*para* ArC), 129.03 (*meta* ArC), 132.05 (*C*H=CH_2), 136.70 (*quat.* ArC), 156.79 (CONH), 171.27 (*C*O_2CH_2) and 175.22 (CO_2H); *m/z* (EI) 307 (7%, M^+), 262 (2, $[M - CO_2H]^+$), 91 (100, $C_7H_7^+$) and 41 (37, $[CH_2\text{-}CH{=}CH_2]^+$).

α-Methyl-β-allyl *N*-Z-(2*S*)-aspartate (55)

To an ice-cold solution of β-allyl N-Z-(2*S*)-aspartate (**54**) (3.0 g, 9.7 mmol) in diethyl ether (10 cm^3) was added dropwise an excess of ethereal diazomethane. The reaction mixture was stirred at for 30 min and then allowed to warm to room temperature over 1 h. Excess diazomethane was removed using a stream of nitrogen and the solvent was then removed under reduced pressure. The crude product was purified by silica

chromatography using petroleum ether/ ethyl acetate (1:1) as the eluant to give the α-methyl ester (**55**) as a colourless oil (330 mg, 68%); $[\alpha]_D$ +12.0 (c 1, CH_2Cl_2); *m/z* (Found: M^+, 321.1207. $C_{16}H_{19}NO_6$ requires 321.1212); υ_{max} (Neat)/cm^{-1} 3403 (NH) and 1746 (CO, acid and ester); δ_H (200 MHz; C^2HCl_3) 2.85 (1 H, dd, 1 of β-CH_2), 3.09 (1 H, dd, 1 of β-CH_2), 3.75 (3 H, s, OCH_3), 4.55 (2 H, d, OCH_2-CH), 4.65 (1 H, m, α-CH), 5.25 (2 H, s, OCH_2Ph), 5.28 (2 H, m, $CH{=}CH_2$), 5.7-5.9 (1 H, m, $CH{=}CH_2$) and 7.35 (5 H, s, ArH); δ_C (50.3 MHz; C^2HCl_3) 36.06 (β-CH_2), 50.83 (α-CH), 53.26 (OCH_3), 66.16 (OCH_2-CH), 67.58 (OCH_2Ph), 119.14 ($CH{=}CH_2$), 128.55 (*ortho* ArC), 128.66 (*para* ArC), 128.98 (*meta* aromatic), 132.08 ($CH{=}CH_2$), 136.58 (*quat.* aromatic), 156.48 (CONH), 170.99 ($\underline{C}O_2CH_2$) and 171.68 ($\underline{C}O_2CH_3$); *m/z* (EI) 321 (8%, M^+), 262 (13, $[M - CO_2CH_3]^+$), 91 (100, $C_7H_7^+$) and 41 (16, $C_3H_5^+$).

α-Methyl *N*-Z-(2*S*)-aspartate (56)

To a stirred solution of α-methyl β-allyl *N*-Z-(2*S*)-aspartate (**55**) (1.20 g, 3.7 mmol) in dichloromethane (30 cm^3), under nitrogen, was added tetrakis (triphenylphosphine) palladium (0) (106 mg, 0.092 mmol), triphenylphosphine (60 mg, 0.233 mmol) and pyrrolidine (0.3 cm^3, 3.6 mmol). The resulting solution was stirred at room temperature for 15 min, washed with 1 mol dm^{-3} hydrochloric acid, dried ($MgSO_4$) and the solvent removed under reduced pressure. The crude product was then purified by silica chromatography using petroleum ether/ ethyl acetate (1:1) as the eluant to give α-methyl *N*-Z-(2*S*)-aspartate (**56**) as a colourless oil, which upon standing became a solid (556 mg, 53%), m.p. 82-83 °C; $[\alpha]_D$ +19.0 (*c* 1, CH_2Cl_2); *m/z* (Found: M^+, 281.0895. $C_{13}H_{15}NO_6$ requires 281.0899); υ_{max} (Nujol)/cm^{-1} 3405 (NH), 3105 (br, $-CO_2H$), 1736 (CO, ester), 1673 (CO, amide I band); δ_H (200 MHz; C^2HCl_3) 2.90 (1 H, m, 1 of β-CH_2), 3.10 (1 H, m, 1 of β-CH_2), 3.75 (3 H, s, OCH_3), 4.65 (1 H, m, α-CH), 5.12 (2 H, s, OCH_2Ph), 5.80 (1 H, d, NH) and 7.38 (5 H, s, ArH); δ_C

(50.3 MHz; ^2H_2O) 36.50 (β-CH_2), 50.82 (OCH_3), 53.44 (α-CH), 67.81 (O*C*H_2Ph), 128.55 (*ortho* ArC), 128.70 (*para* ArC), 129.05 (*meta* ArC), 136.44 (*quat.* ArC), 158.84 (CONH), 171.88 (*C*O_2CH_3) and 178.28 (CO_2H); *m/z* (EI) 281 (7%, M^+), 222 (6, $[M - CO_2CH_3]^+$) and 91 (100, $C_7H_7^+$).

α-Methyl (2*S*)-aspartate (57)

To a stirred solution of α-methyl *N*-Z-(2*S*)-aspartate (56) (820 mg, 2.91 mmol) in methanol (20 cm^3) was added 10% palladium on activated carbon (160 mg) and the reaction vessel flushed with hydrogen gas. The resultant suspension was stirred vigorously under an atmosphere of hydrogen for 12 h. The mixture was then filtered through a prewashed celite pad, the solvent removed under reduced pressure, and the crude product recrystallised from water/ acetone, and then from water/ethanol to give the pure α-methyl ester (57) as a white solid (373 mg, 87%), m.p. 169-71 °C (lit.,[119] 167 °C); $[\alpha]_D$ 38.40 (*c* 0.5, H_2O) {lit.,[119] $[\alpha]_D^{28}$ +37.60 (*c* 0.5, H_2O)}; (Found: C, 40.3; H, 6.05; N, 8.85. Calc. for $C_5H_9NO_4$: C, 40.8; H, 6.15; N, 9.5%); δ_H (200 MHz; ^2H_2O) 2.72 (2 H, m, β-CH_2), 3.79 (3 H, s, OCH_3) and 4.23 (1 H, m, α-CH); δ_C (50.3 MHz; ^2H_2O), 38.61 (β-CH_2), 53.10 (α-CH), 53.36 (OCH_3), 173.28 (*C*O_2CH_3), 178.93 (CO_2H); *m/z* (CI) 148 (100%, $[M + H]^+$), 132 (21, $[M - CH_3]^+$), 102 (17, $[M - CO_2H]^+$) and 88 (11, $[M - CO_2CH_3]^+$).

(2*S*)-Aspartic acid β-methyl ester hydrochloride (58)

To a stirred solution of (2*S*)-aspartic acid (10.0 g, 75.2 mmol) in dry methanol (45 cm^3) at -10 °C was added thionyl chloride (5.49 cm^3, 75.2 mmol). The solution was allowed to warm to room temperature and then after 30 min the solvent was removed under reduced pressure to half the original volume. Diethyl ether (130 cm^3)

was added, and the resulting white crystalline solid was filtered, washed with cold diethyl ether and dried to give the β-ester (**58**) (11.0 g, 80%), m.p. 189-90 °C (lit.,[122] 204 °C); $[\alpha]_D$ +12.9 (*c* 1, 1:3 ethanol-water){lit.,[123] $[\alpha]_D$ +12.4 (*c* 1, 1:3 ethanol-water)}; (Found: C, 32.5; H, 5.32; N, 7.45. Calc. for $C_5H_{10}NO_4Cl$: C, 32.7; H, 5.5 and N, 7.65%); δ_H (200 MHz; ^2H_2O) 3.06 (2 H, d, β-CH_2), 3.67 (3 H, s, OCH_3) and 4.35 (1 H, dd, α-CH); δ_C (50.3 MHz; ^2H_2O) 38.82 (β-CH_2), 52.88 (α-CH), 55.77 (OCH_3), 173.81 (CO_2CH_3) and 174.70 (CO_2H).

(2*S*)-Aspartic acid β-methyl ester (59)

(2*S*)-Aspartic acid β-methyl ester hydrochloride (**58**) (6.81 g, 37 mmol) and propylene oxide (9.2 cm^3) were refluxed in dry ethanol (140 cm^3) for 3 h. On cooling, the precipitate was filtered, washed with diethyl ether and then recrystallised with ethanol/ diethyl ether to give a white solid (4.9 g, 90%), m.p. 190 °C (lit.,[125] 194-195 °C); (Found: C, 40.75; H, 6.4; N, 9.5. Calc. for $C_5H_9NO_4$: C, 40.8; H, 6.15; N, 9.5%); υ_{max} (Nujol)/cm^{-1} 3199(NH), 1761 (CO, acid) and 1741 (CO, ester); δ_H (200 MHz; ^2H_2O) 2.99 (2 H, m, β-CH_2), 3.69 (3 H, s, OCH_3) and 4.0 (1 H, dd, α-CH); δ_C (50.3 MHz; ^2H_2O) 37.27 (β-CH_2), 53.47 (α-CH), 55.49 (OCH_3), 175.34 (CO_2CH_3) and 175.68 (CO_2H); *m/z* (CI) 148 (100%, $[M + H]^+$), 132 (1, $[M - CH_3]^+$) and 102 (2, $[M - CO_2H]^+$)

Dimethyl (2*S*)-aspartate hydrochloride (60)

To a stirred solution of (2*S*)-aspartic acid (**52**) (13.3 g, 0.10 mol) in dry methanol (70 cm^3) was added dropwise thionyl chloride (10.19 cm^3, 0.14 mol) at 0 °C. The ice bath was removed and the solution was allowed to stir at room temperature for 45 h and then concentrated under reduced pressure. The residual oil was triturated with

diethyl ether and the hydrochloride salt was collected by filtration. Recrystallisation from ethanol/ diethyl ether gave the hydrochloride as a crystalline white solid (15.9 g, 80%), m.p. 100-101 °C (lit.,[125] 114-15 °C); $[\alpha]_D$ +12.3 (*c* 1, water); (Found: C, 36.95; H, 6.35; N, 7.05. Calc. for $C_6H_{12}NClO_4$: C, 36.45; H, 6.1; N, 7.1%); υ_{max} (Nujol)/cm^{-1} 3472 (NH) and 1741 (CO, ester); δ_H (200 MHz; ^2H_2O) 3.18 (2 H, t, β-CH_2), 3.65 (3 H, s, β-OCH_3), 3.89 (3 H, s, α-OCH_3) and 4.45 (1 H, t, α-CH); δ_C (50.3 MHz; ^2H_2O) 36.56 (β-CH_2), 52.06 (α-CH), 55.90 (β-OCH_3), 56.85 (α-OCH_3), 172.20 (β-CO_2CH_3) and 174.48 (α-CO_2CH_3); *m/z* (EI) 162 (22%, $[M - Cl]^+$), 102 (100, $[M - Cl - CO_2CH_3 - H]^+$) and 59 (25, $CO_2CH_3^+$).

(2*S*)-2-Amino succinic diamide hydrochloride (61)

Dimethyl-(2*S*)-aspartic acid hydrochloride (**60**) (1.00 g, 50.5 mmol) was dissolved in dry methanol (30 cm^3). Ammonia gas was passed into the solution at 0 °C. The solution was then placed in a tightly stoppered vessel and allowed to warm to room temperature. After 45 h, the solvent was removed under reduced pressure to give the crude product. Recrystallisation from water/acetone gave the diamide as a white solid (830 mg, 98%), m.p. 214 °C (decomp.); $[\alpha]_D$ +8.25 (*c* 0.4, water); *m/z* (Found: $[M + H]^+$, 132.0770. $C_4H_{10}N_3O_2$ requires 132.0773); υ_{max} (Nujol)/cm^{-1} 3472 & 3384 (NH), 1692 (CO, amide I band) and 1634 (NH bending); δ_H (200 MHz; ^2H_2O) 2.70-3.05 (2 H, m, β-CH_2) and 4.25 (1 H, t, α-CH); δ_C (50.3 MHz; ^2H_2O) 38.19 (β-CH_2), 52.65 (α-CH), 174.52 (β-$CONH_2$) and 176.00 (α-$CONH_2$); *m/z* (CI) 132 (26%, $[M + H]^+$), 115 (6, $[M - NH_2]^+$), 73 (5, $H_2NCHCONH_2^+$), 87 (6, $[M - CONH_2]^+$) and 57 (100, $[CH_2CONH_2 + H]^+$).

β-Allyl *N*-Z-(2*S*)-aspartamide (62)

To a solution of β-allyl *N*-Z-(2*S*)-aspartate (**54**) (200 mg, 0.65 mmol) in dry THF ($10\ cm^3$) was added N-methyl morpholine ($71\ mm^3$, 0.65 mmol) and the solution cooled to -15 °C. Isobutyl chloroformate ($88\ mm^3$, 0.65 mmol) was added with stirring and the solution left to stir at -15 °C for 30 min. The solution was filtered into a dry methanolic ammonia solution at -10 °C and stirring continued for 10 min. The solution was then allowed to warm to room temperature and stirred for a further 15 h. The solvent was removed under reduced pressure and the crude product was purified by silica chromatography using petroleum ether/ ethyl acetate (1:2) to give β-allyl *N*-Z-(2*S*)-aspartamide (**62**) as a white solid (118 mg, 62%), m.p. 86 °C; $[\alpha]_D$ +19.3 (*c* 1, CH_2Cl_2); *m/z* (Found: M^+, 306.1208. $C_{15}H_{18}N_2O_5$ requires 306.1215); υ_{max} (Nujol)/cm^{-1} 3423 & 3306 (NH), 1710 (CO, ester), 1673 (CO, amide I band) and 1625 (C=C); δ_H (200 MHz; C^2HCl_3) 2.71 (1 H, m, 1 of β-CH_2), 3.08 (1 H, m, 1 of β-CH_2), 4.65 (3 H, m, α-CH and OCH_2-CH), 5.19 (2 H, s, OCH_2Ph), 5.21-5.40 (2 H, m, CH=CH_2), 5.60-6.15 (2 H, m, *CH*=CH_2 and CONH), 6.52 (2 H, br s, $CONH_2$) and 7.4 (5 H, s, ArH); δ_C (50.3 MHz; C^2HCl_3) 36.62 (β-CH_2), 51.29 (α-CH), 66.24 (OCH_2-CH), 67.78 (OCH_2Ph), 119.21 (CH=CH_2), 128.65 (*para* ArC), 128.81 (*ortho* ArC), 129.08 (*meta* ArC), 132.10 (*C*H=CH_2), 136.46 (*quat.* ArC), 156.65 (CONH), 171.90 (CO_2CH_2) and 173.72 ($CONH_2$); *m/z* (EI) 306 (2%, M^+), 91 (100, $C_7H_7^+$) and 41 (8, $C_3H_5^+$).

***N*²-Benzyloxycarbonyl (2*S*)-2-amino succinic acid 1-amide (63)**

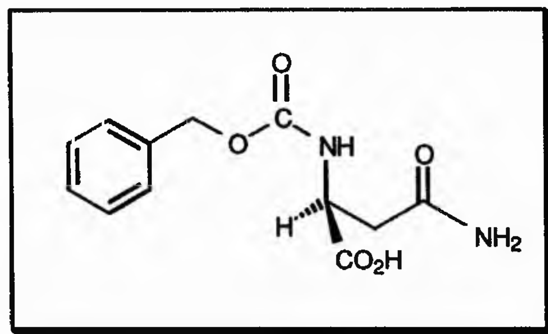
To a solution of β-allyl *N*-Z-(2*S*)-aspartamide (**62**) (2.50 g, 8.5 mmol) in dichloromethane ($30\ cm^3$) under nitrogen was added tetrakis (triphenylphosphine) palladium (0) (245 mg, 0.21 mmol), triphenylphosphine (140 mg, 0.53 mmol) and pyrrolidine ($0.4\ cm^3$, 4.78 mmol). The resulting white thick solution was stirred for

10 min, washed with 1 mol dm^{-3} hydrochloric acid, dried ($MgSO_4$), and concentrated under reduced pressure to give the crude product. Purification by silica chromatography using ethyl acetate/ petroleum ether (2:1) as the eluant then gave pure N^2-benzyloxycarbonyl (2*S*)-2-amino succinic acid 1-amide (**63**) (1.5 g, 66%), m.p. 159-160 °C; *m/z* (Found: [M + H]$^+$, 267.0986. $C_{12}H_{15}N_2O_5$ requires 267.0980); υ_{max} (Nujol)/cm^{-1} 3403 & 3325 (NH) and 1697 (CO, amide I band); δ_H (200 MHz; $C^2H_3O^2H$) 2.78 (2 H, m, β-CH_2), 4.55 (1 H, m, α-CH), 5.19 (2 H, s, C*H*$_2$Ph) and 7.35 (5 H, s, ArH); δ_C (75.4 MHz; d_6-DMSO) 36.84 (β-CH_2), 51.74 (α-CH), 65.99 (O*C*H$_2$Ph), 128.30 (*para* ArC), 128.37 (*ortho* ArC), 128.91 (*meta* ArC), 137.58 (*quat.* ArC), 156.44 (CONH), 172.46 ($CONH_2$) and 173.40 (CO_2H); *m/z* (CI) 267 (100%, [M + H]$^+$), 223 (3, [M + H - $CONH_2$]$^+$) and 91 (12, $C_7H_7^+$).

(2*S*)-2-amino succinic acid 1-amide (Isoasparagine) (64)

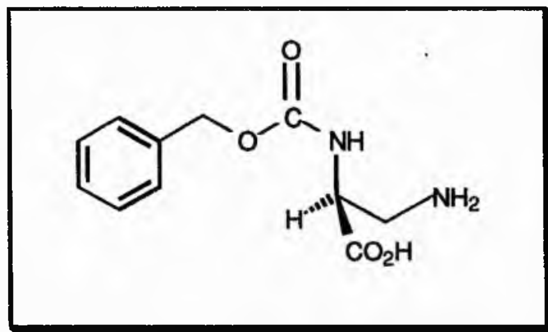
To a solution of N^2-benzyloxycarbonyl (2*S*)-2-amino succinic acid 1-amide (666 mg, 2.5 mmol) in methanol (30 cm^3) was added 10% palladium on activated carbon (120 mg) and the reaction vessel flushed with hydrogen gas. The resultant suspension was stirred vigorously under an atmosphere of hydrogen for 16 h. The mixture was then filtered through a prewashed celite pad. The pad was washed with methanol (15 cm^3), water (20 cm^3) and the solvent removed under reduced pressure to give the crude amide (**64**). Recrystalization from water/ethanol then gave the pure (2*S*)-2-amino succinic acid 1-amide (**64**) (250 mg, 75%), m.p. 214-216 °C (decomp.) {lit.,[118] m.p. 212-214 °C (decomp.)}; $[\alpha]_D$ +16.7 (*c* 1.8, 0.1 mol dm^{-3} HCl) {lit.,[118] $[\alpha]_D^{26}$ +15.4 (*c* 1.8, 0.1 mol dm^{-3} HCl)}; (Found: C, 31.75; H, 6.7; N, 18.15. Calc. for $C_4H_8N_2O_3.H_2O$: C, 32.0; H, 6.7; N, 18.7%); δ_H (200 MHz; ^2H_2O) 2.65 (2 H, m, β-CH_2) and 4.10 (1 H, m, α-CH); δ_C (50.3 MHz; ^2H_2O) 39.52 (β-CH_2), 53.32 (α-CH), 174.76 ($CONH_2$) and 178.99 (CO_2H); *m/z* (CI) 133 (100%, [M + H]$^+$), 115 (5, [M - NH_3]$^+$), 88 (2, [M - CO_2H]$^+$) and 73 (9, [M - CH_2CO_2H]$^+$).

N-Benzyloxycarbonyl-(2S)-asparagine (68)



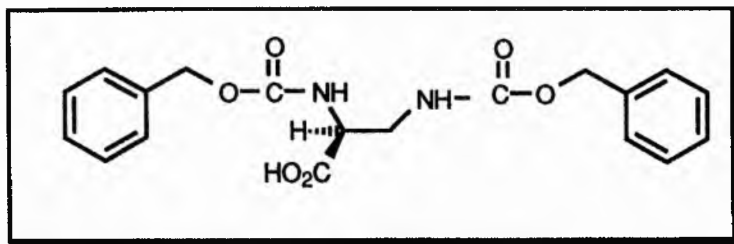
(2*S*)-Asparagine (10.0 g, 75.8 mmol) was dissolved in water (350 cm^3) and the pH of the solution adjusted to 8 with the slow addition of sodium bicarbonate (15.9 g, 190 mmol). The solution was then vigorously stirred while benzylchloroformate (12.9 cm^3, 91 mmol) was added in five portions over 20 min. After overnight stirring, the solution was extracted with ether (2 x 75 cm^3) and the aqueous phase was acidified with 12 mol dm^{-3} hydrochloric acid. The resultant precipitate was filtered and recrystallised from ethanol/ether to give white shiny needle shaped crystals (15.8 g, 78%), m.p. 160-62 °C (decomp.) {lit.,[137] 165 °C (decomp.)}; $[\alpha]_D$ +6.4 (*c* 1.6, AcOH) {lit.,[137] $[\alpha]_D$ +7.8 (*c* 1.6, AcOH)}; υ_{max} (Nujol)/cm^{-1} 3337 (N-H) and 1669 (C=O); δ_H (200 MHz; ^2H_2O), 2.40-2.80 (2 H, m, β-CH_2), 4.25 (1 H, m, α-CH), 5.1 (2 H, s, CH_2Ph) and 7.37 (5 H, s, ArH); δ_C (74.46 MHz; d_6-DMSO), 36.7 (β-CH_2), 50.6 (α-CH), 65.4 (CH_2Ph), 127.6, 127.7 (*ortho*, *para* ArC), 128.2 (ArC), 155.7 (ArCO), 171.1 (CO_2H) and 172.9 ($CONH_2$); *m/z* (EI) 249 (72%, $[M - NH_3]^+$) and 91 (100, $C_7H_7^+$).

N^2-Benzyloxycarbonyl-(2*S*)-2,3-diaminopropanoic acid (69)



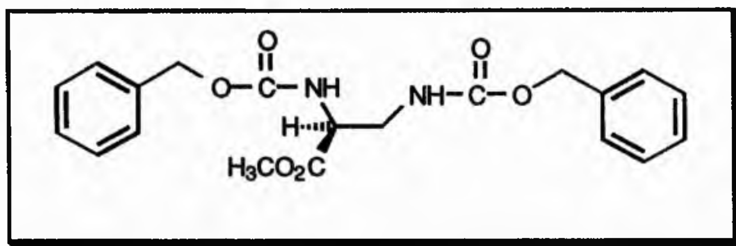
N-Benzyloxycarbonyl-(2*S*)-asparagine (**68**) (6.0 g, 22.6 mmol) was added to the stirred solution of *bis* [trifluoroacetoxy]-phenyl iodine (14.6 g, 33.9 mmol) in *N,N*-dimethyl formamide/water (180 cm^3, 1;1 v/v) at room temperature. After 15 min, pyridine (3.6 cm^3, 45.3 mmol) was added and stirring continued overnight. The solvent was removed by distillation under reduced pressure and the resulting oil dissolved in water (60 cm^3). The solution was extracted with ether (2 x 70 cm^3) and the aqueous phase concentrated under reduced pressure to give a yellow oil. Trituration with ethyl acetate resulted in the precipitation of the product, which was then recrystallised from water to give a white shiny crystals (3.42 g, 64%), m.p. 230-232 °C {lit.,[129] m.p. 228-230 °C (decomp.)}; $[\alpha]_D$ -6.9 (*c* 0.4, 1 mol dm^{-3} NaOH) {lit.,[129] $[\alpha]_D$ -7.8 (*c* 0.4, 1 mol dm^{-3} NaOH)}; *m/z* (Found: M^+, 238.0957. Calc. for $C_{11}H_{14}N_2O_4$: 238.0953); υ_{max} (Nujol)/cm^{-1} 3302 (N-H) and 1694 (C=O); δ_H (200 MHz; ^2H_2O) 2.65-3.0 (2 H, m, β-CH_2), 3.95 (1 H, q, α-CH), 5.1 (2 H, s, CH_2Ph) and 7.40 (5 H, s, ArH); δ_C (74.46 MHz; ^2H_2O + NaOD) 44.80 (β-CH_2), 56.02 (α-CH), 63.99 (OCH_2Ph), 127.65, 128.01 & 128.93 (*ortho* and *para* ArC), 140.48 (q ArC), 166.06 (CONH) and 179.69 (CO_2H); *m/z* (EI) 238 (4%, M^+) and 91 (100, $C_7H_7^+$).

$N^{2,3}$-Benyloxycarbonyl-(2*S*)-2,3-diaminopropanoic acid (70)



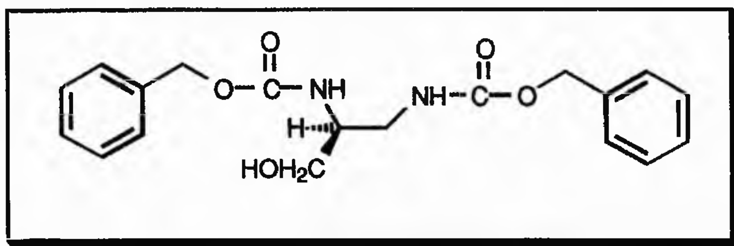
Into boiling water (450 cm^3) was added N^2-benzyloxycarbonyl-(2*S*)-2,3-diamino propanoic acid (3.0 g, 12.6 mmol). The clear solution was allowed to cool to 60 °C and then $NaHCO_3$ (2.64 g, 31.5 mmol) was added carefully untill the pH of the solution was 8. Benzylchloroformate (2.15 cm^3, 15.1 mmol) was added to the vigorously stirring solution over 30 min at 60 °C, and stirring continued at room temperature for 6 h. The mixture was extracted with ether (2 x 75 cm^3), the aqueous phase was acidified with 12 mol dm^{-3} HCl and again extracted with ether (3 x 60 cm^3). The ethereal layers were combined, dried ($MgSO_4$) and the solvent was removed under reduced pressure to give an oil. The crude was purified by silica column chromatography using ethyl acetate/pet.ether (1:1) as the eluant to give a white solid (4.29 g, 92%), m.p. 96-97 °C; $[\alpha]_D$ +40 (*c*, 0.33, CH_2Cl_2); *m/z* (Found: $[M + H]^+$, 373.1387. $C_{19}H_{21}N_2O_6$ requires 373.1399); δ_H (200 MHz; C^2HCl_3) 3.60 (2 H, m, β-CH_2), 4.45 (1 H, m, α-CH), 5.03-5.07 (4 H, d, 2 x CH_2Ph), 5.82 (1 H, t, CH_2NHCO), 6.25-6.28 (1 H, d, CO_2NHCH), 7.28-7.30 (10 H, d, ArH) and 8.17 (1 H, br, s, CO_2H); δ_C (50.3 MHz; C^2HCl_3) 42.97 (β-CH_2), 55.15 (α-CH), 67.78 (CH_2Ph), 128.64, 128.74, & 129.04 (*ortho* and *para* ArC), 136.78 (q ArC), 157.16 (CONH), 158.05 (CONH) and 173.84 (CO_2H); *m/z* (CI) 373 (29%, $[M + H]^+$) and 329 (100, $[M + H - CO_2]^+$).

$N^{2,3}$-Benzyloxycarbonyl-(2*S*)-2,3-diaminopropanoic acid methyl ester (75)



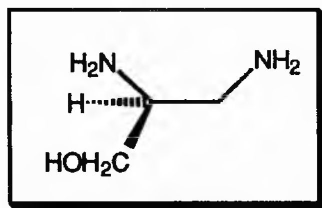
To a stirred solution of $N^{2,3}$-Benzyloxycarbonyl-(2*S*)-2,3-diamino propanoic acid, (**70**) (500 mg, 1.34 mmol) in dry methanol (30 cm^3) was added dropwise thionyl chloride (0.12 cm^3) at 0 °C. The ice bath was removed and the solution allowed to warm to room temperature and then refluxed for 3 h. The solvent was then removed under reduced pressure to give the crude product. Purification by silica column chromatography using pet. ether /ethyl acetate (1:1) as the eluant gave the pure ester (**75**) as a white crystalline solid (300 mg, 58%), m.p. 71-72 °C; $[\alpha]_D$ +12.0 (*c* 0.5, CH_2Cl_2); (Found: C, 61.9; H, 5.8; N, 7.15. $C_{20}H_{22}N_2O_6$ requires C, 62.2; H, 5.75; N, 7.25%); δ_H (300 MHz; C^2HCl_3) 3.61-3.63 (2 H, d, β-CH_2), 3.72 (3 H, s, OCH_3), 4.42-4.44 (1H, d, α-CH), 5.07-5.10 (4 H, d, CH_2Ph), 5.16 (1 H, s, br, CH_2NHCO), 5.78 (1 H, s, br, CO_2NHCH) and 7.32-7.34 (10 H, d, ArH); δ_C (74.46 MHz; C^2HCl_3) 42.90 (β-CH_2), 52.95 (OCH_3), 54.95 (α-CH), 67.30 & 67.4 (2 x CH_2Ph), 128.53, 128.60 & 128.94 (ArC), 136.63 & 136.77 (ArC), 156.70 (NHCO), 157.43 (CONH) and 171.54 (CO_2CH_3); *m/z* (EI) 387 (18%, $[M + H]^+$), 327 (8, $[M - CO_2CH_3]^+$), 251 (8, $[M - C_6H_6CO_2]^+$), 223 (23, $[M + H - C_6H_5CO_2NHCH_2]^+$) and 91 (100, $C_7H_7^+$).

$N^{2,3}$-Benzyloxycarbonyl -(2*S*)-2,3-diamino propane-1-ol (71)



To a stirred solution of $N^{2,3}$-Benzyloxycarbonyl-(2*S*)-2,3-diamino propanoic acid (3.0 g, 8.06 mmol) in dry THF (60 cm^3) at -30 °C was added N-methylmorpholine (0.88 cm^3, 8.06 mmol) and isobutyl chloroformate (1.04 cm^3, 8.06 mmol) respectively. A white precipitate formed very quickly and after 30 min the mixture was filtered into a solution of $NaBH_4$ (182.84 mg, 4.84 mmol) in dry THF (20 cm^3) at -30 °C. The reaction was stirred at this temperature for 3 h and then left stir overnight at room temperature. Water (30 cm^3) was addded carefully to the reaction mixture and the solution was concentrated under reduced pressure until the water started to distill off. The aqueous solution was extracted with ether (3 x 70 cm^3) and the ethereal layers were combined, washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure to give the crude product as a solid. Purification by silica coloumn chromatography using ethyl acetate/ pet. ether (1:1) as the eluant gave a white solid (1.96 g, 68%), m.p. 105-106 °C; $[\alpha]_D$ +8.2 (*c* 1, CH_2Cl_2); (Found: C, 6.3; H, 63.35; N, 7.75%. Calc. for $C_{19}H_{22}N_2O_5$: C, 6.2; H, 63.7 and N, 7.8%); *m/z* (Found: [M + H]$^+$, 359.1608. Calc. for $C_{19}H_{23}N_2O_5$: 359.1607); δ_H (300 MHz; C^2HCl_3) 3.20-3.70 (6 H, m, β-CH_2, α-CH & CH_2OH), 5.07-5.10 (4 H, d, 2 x CH_2Ph), 5.40-5.45 (1 H, t, N*H*CH_2), 5.49-5.51 (1 H, d, N*H*CH) and 7.33-7.34 (10 H, d, ArH); δ_C (74.46 MHz; C^2HCl_3) 40.69 (β-CH_2), 52.56 (α-CH), 61.38 (CH_2OH), 66.77 & 67.15 (2 x *C*H_2Ph), 127.90, 128.00, 128.05, 128.19, 128.40 & 128.45 (ArC), 135.90 & 136.08 (q ArC), and 156.21 & 157.91 (2 x CONH); *m/z* (CI) 359 (41%, [M + H]$^+$), 341 (3, [M + H - H_2O]$^+$), 251 (100, [M - $C_6H_6CH_2O$]$^+$), 207 (6, [M + H - $C_6H_5CO_2NH$]$^+$) and 91 (37, $C_7H_7^+$).

2,3-Diaminopropane-1-ol (66)



To a solution of $N^{2,3}$-Benzyloxycarbonyl-(2*S*)-2,3-diaminopropane-1-ol (1.42 g, 3.96 mmol) in methanol (70 cm^3) was added 10% palladium on activated carbon (500 mg) and the reaction vessel flushed with hydrogen gas. The resultant mixture was stirred vigorously under an atmosphere of hydrogen and the progress of the reaction followed by t.l.c. After 16 h, the solution was filtered through prewashed celite pad, and the celite pad washed with methanol (20 cm^3). The filtrate was concentrated under reduced pressure to give 2,3-diamino-1-propanol as an oil (330 mg, 93%), $[\alpha]_D$ +9.5 (*c* 2, MeOH); δ_H (200 MHz; ^2H_2O) 2.45-2.93 (3 H, m, α-CH & β-CH_2) and 3.38-3.63 (2 H, m, CH_2OH); δ_C (50.3 MHz; ^2H_2O) 46.21 (β-CH_2), 56.16 (α-CH) and 66.81 (CH_2OH); *m/z* (EI) 92 (76%, $[M + 2H]^+$), 72 (7, $[M - H_2O]^+$), 60 (100, $C_2H_6NO^+$) and 59 (23, $[M - CH_2OH]^+$).

2,3-Diaminopropane-1-ol dihydrochloride salt (73)

2,3-Diaminopropane-1-ol **(66)** (300 mg, 3.32 mmol) was dissolved in methanol (15 cm^3) and the solution cooled to at 0 °C. HCl gas was bubbled through the solution for 10 min. The solvent was then removed reduced under pressure to give the crude hydrochloride salt, which was recrystallisation from water/ ethanol to give a pale yellow solid (240 mg, 44%), m.p. 180-81 °C (lit.,[127] 172-74 °C); $[\alpha]_D$ +23.93 (*c* 0.33, H_2O); *m/z* (Found: $[M + H - 2HCl]^+$, 91.0869. $C_3H_{11}N_2O$ requires 91.0871); δ_H (200 MHz; ^2H_2O) 3.33 (2 H, m, CH_2NH_2) 3.67-3.81 (1 H, m, α-CH) and 3.82-

4.0 (2 H, m, CH_2OH); δ_C (50.3 MHz; ^2H_2O) 41.31 (CH_2NH_2), 52.88 (α-CH) and 61.93 (CH_2OH); *m/z* (CI) 129 (1.5%, $[M + H - Cl]^+$) and 91 (100, $[M + H - 2HCl]^+$].

2,3-Diaminopropyl hydrogen sulfate hydrochloride (74)

To a cooled suspension of 2,3-diamino propanol (**66**) (50 mg, 0.55 mmol) in dichloromethane (15 cm^3) was added dropwise, and then more rapidly a cooled mixture of chlorosulfonic acid (0.05 cm^3, 0.83 mmol) in dichloromethane (1 cm^3). The ice bath was removed and the reaction mixture was allowed to stir at room temperature for 45 h. The solvent was removed under reduced pressure to give the 2,3-diaminopropyl hydrogensulfate as a hydrochloride salt. Recrystallisation from water/ ethanol gave a white solid (80 mg, 59%), m.p. 55-60 °C; $[\alpha]_D$ +9.57 (*c* 1.17, H_2O); δ_C (50.3 MHz; ^2H_2O) 40.57 (β-CH_2), 50.72 (α-CH) and 67.44 (CH_2OSO_3H); *m/z* (CI) 171 (3.5%, $[M - 2HCl]^+$), 111 (30, $CH_2OSO_3H^+$), 97 (27, OSO_3^+), 73 (100, $[M - 2HCl - OSO_3H]^+$) and 60 (11, $[M + H - HCl - CH_2OSO_3H]^+$).

α-Isopropyl-*N*-trifluroacetyl-(2*S*)-aspartate (77)

To a suspension of (2*S*)-aspartic acid (5 g, 37 mmol) in dry THF (60 cm^3) at 0 °C, under an atmosphere of nitrogen, was added dropwise trifluoroacetic anhydride (21.18 cm^3, 0.15 mmol) over 30 min. The reation mixture was allowed to stir at 0 °C for 1 h and then at room temperature for a further 2 h during which time dissolution was completed. The solvent was removed under reduced pressure to give the anhydride as a white solid. The anhydride was treated with dry isopropanol (70 cm^3) and the solution stirred at 40 °C for 12 h under nitrogen. The solvent was removed under reduced pressure to give the crude isopropyl ester. Recrystallisation from diethyl ether/ pet. ether gave pure α-*iso*propyl-*N*-trifluroacetyl-(2*S*)-aspartate (9.8 g, 97%);

m.p. 98.2 °C (lit.,[137] 88-90 °C); $[\alpha]_D$ +40.6 (*c* 0.5, CH_2Cl_2); (Found; C, 39.9; H, 4.65; N, 5.1. Calc. for $C_9H_{12}NO_5F_3$: C, 39.85; H, 4.46; N, 5.15%); *m/z* (Found: [M + H]$^+$, 272.0736. Calc. for $C_9H_{13}NO_5F_3$: 272.0745); υ_{max} (Nujol)/cm^{-1} 3320 (NH str), 1760 (ester C=O), 1745 (acid C=O) and 1710 (trifluoroacetyl C=O); δ_H (200 MHz; C^2HCl_3) 1.21-1.30 (6 H, dd, 2 x $CH(CH_3)_2$), 2.93-3.23 (2 H, dd, β-CH_2), 4.78 (1 H, m, α-CH), 5.11 (1 H, m, $CH(CH_3)_2$), and 7.36 & 7.37 (1 H, dd, NH); δ_C (50.3 MHz; C^2HCl_3) 21.40 &21.54 (2 x $CH(CH_3)_2$), 35.25 (β-CH_2), 48.88 (α-CH), 71.03 ($CH(CH_3)_2$), 168.46 (CONH), 168.49 ($CO_2{}^iPr$), and 175.73 (CO_2H); *m/z* (EI) 272 (42%, [M + H]$^+$), 226 (13, [M - CO_2H]$^+$), 212 (53, [M - $OCH(CH_3)_2$]$^+$), 184 (100, [M - $CO_2CH(CH_3)_2$]$^+$), 166 (66, [M - $CO_2CH(CH_3)_2$ - H_2O]$^+$), 139 (90, [M - $CO_2CH(CH_3)_2$ - CO_2H]$^+$) and 43 (98, $C_3H_7{}^+$).

α-*Iso*propyl-*N*-trifluoroacetyl-(2*S*)-aspartyl-β-acid chloride (78)

To a stirred solution of α-isopropyl-*N*-trifluroacetyl-(2*S*)-aspartic acid (5 g, 18.44 mmol) in dry dichloromethane (20 cm^3) was added distilled thionyl chloride (5.36 cm^3, 73.6 mmol) and the mixture was reflux for 12 h under nitrogen. The solvent was then removed under reduced pressure to yield the β-acid chloride (4.05 g, 76%), m.p. 120-123 °C (lit.,[138] 123-5 °C); $[\alpha]_D$ +65.4 (*c* 0.63, CH_2Cl_2) {lit.,[138] $[\alpha]_D$ +65.8 (*c* 0.63, CH_2Cl_2)}; υ_{max} (Nujol)/cm^{-1} 3327 (NH str), 1806 (acid chloride C=O), 1733 (ester C=O), and 1710 (trifluoroacetyl C=O); δ_H (200 MHz; C^2HCl_3) 1.3 (6 H, dd, 2 x $CH(CH_3)_2$), 3.62 (2 H, m, β-CH_2), 4.7 (1 H, m, α-CH), 5.15 (1 H, m, $CH(CH_3)_2$) and 7.36 & 7.37 (1 H, br., NH); δ_C (50.3 MHz; C^2HCl_3) 21.46 & 21.56 (2 x $CH(CH_3)_2$), 47.4 (β-CH_2), 49.4 (α-CH), 71.65 ($CH(CH_3)_2$), 157.40 (CONH, *J* 37), 167.79 ($CO_2{}^iPr$) and 172.35 (COCl).

$\it{Iso}$propyl 5-diazo-4-oxo-*N*-trifluoroacetyl-(2*S*)-norvalinate (79)

A solution of *iso*propyl *N*-trifluoroacetyl-(2*S*)-aspartyl-β-chloride (**78**) (3 g, 10.35 mmol) in diethyl ether (40 cm^3) and tetrahydrofuran (10 cm^3) was added dropwise to excess ethereal diazomethane (175 cm^3) over 30 min, at 0 °C with stirring. The solution was allowed to warm to room temperature during 90 min after which time excess diazomethane was removed using a stream of nitrogen. The solvent was then removed under reduced pressure to give a pale yellow solid which was recrystallised from diethyl ether/ cyclohexane to give yellow crystals of the diazoketone (2.90 g, 95%), m.p. 91-93 °C; $[\alpha]_D$ +22.8 (*c* 1, CH_2Cl_2); *m/z* (Found: $[M + H]^+$ 296.0856. $C_{10}H_{13}N_3O_4F_3$ requires 296.0858); υ_{max} (Nujol)/cm^{-1} 3316 (NH str), 2126 (diazoketone CHN_2), 1746 (ester C=O), 1710 (trifluoroacetyl C=O) and 1644 (diazo C=O); δ_H (300 MHz; C^2HCl_3) 1.21-1.25 (6 H, dd, 2 x $CH(CH_3)_2$), 2.85-3.09 (2 H, dd, β-CH_2), 4.69 (1 H, m, α-CH), 5.05 (1 H, m, $CH(CH_3)_2$), 5.32 (1 H, s, CHN_2) and 7.38-7.41 (1 H, d, NH); δ_C (74.46 MHz; C^2HCl_3) 21.48, 21.56 (2 x $CH(CH_3)_2$), 40.28 (β-CH_2), 49.26 (α-CH), 55.60 (CHN_2), 70.48 {$OCH(CH_3)_2$}, 157.39 (CF_3CONH), 168.75 {$COCH(CH_3)$} and 191.46 ($COCHN_2$); *m/z* (EI) 296 (44%, $[M + H]^+$), 268 (32, $[M + H - N_2]^+$), 226 (100, $[M - COCHN_2]^+$) and 113 (35, $[M + H - CF_3CONH)]^+$).

$\it{Iso}$propyl 4-oxo-*N*-trifluoroacetyl-(2*S*)-norvalinate (80)

Aqueous HI (55%; excess) was added dropwise to a stirred solution of the diazoketone (**79**) (2.80 g, 9.5 mmol) in dichloromethane (40 cm^3). After 15 min the organic layer was washed with water (2 x 10 cm^3), dilute aqueous sodium thiosulfate (until colourless), water (5 cm^3) and then dried (Na_2SO_4). The solvent was removed under reduced pressure to yield the ketone. Recrystallised from diethyl ether/pet. ether gave the ketone as white crystals (2.0 g, 78%), m.p. 50-52 °C; (Found: C, 44.45; H, 5.3;

N, 5.22. $C_{10}H_{15}NO_4F_3$ requires C, 44.6; H, 5.25; N, 5.2%); $[\alpha]_D$ +37.2 (*c* 0.5, CH_2Cl_2); *m*/*z* (Found: $[M + H]^+$, 270.0962. $C_{10}H_{15}NO_4F_3$ requires 270.0953); υ_{max} (Nujol)/cm^{-1} 3307 (NH str), 1739 (ester C=O), and 1710 (trifluoroacetyl C=O and ketone C=O); δ_H (200 MHz; C^2HCl_3) 1.19-1.27 (6 H, dd, 2 x $CH(CH_3)_2$), 2.18 (3 H, s, CH_3), 2.95-3.35 (2 H, ABX, β-CH_2), 4.63-4.71 (1 H, q, α-CH), 4.99-5.12 {1 H, sp, OC*H*$(CH_3)_2$} and 7.38-7.41 (1 H, d, NH); δ_C (74.76 MHz; C^2HCl_3) 21.45 & 21.52 (2 x CH$(C H_3)_2$), 29.77 (CO*C*H_3), 43.90 (β-CH_2), 48.73 (α-CH), 70.43 (O*C*H$(CH_3)_2$), 168.83 (*C*$O_2{}^iPr$)) and 206.39 (*C*OCH_3); *m*/*z* (EI) 270 (7%, $[M + H]^+$), 226 (16, $[M - COCH_3]^+$), 212 (13, $[M - CH_2COCH_3]^+$), 210 (18, $[M - OCH(CH_3)_2]^+$), 182 (36, $[M - CO_2CH(CH_3)_2]^+$), 59 (11, $[OCH(CH)_2]^+$) and 43 (100, $[CH(CH_3)_2]^+$).

4-Oxo-(2*S*)-norvaline (92)

To a solution of *Iso*propyl 4-oxo-*N*-trifluoroacetyl-(2*S*)-norvalinate (**80**) (100 mg, 0.37 mmol) in methanol (0.91 cm^3) was added 1 mol dm^{-3} NaOH (1 cm^3). The resulting mixture was stirred at room temperature for 1 h and then after the addition of 1 mol dm^{-3} HCl (1 cm^3) the solvent was removed under reduced pressure to give crude **92.** δ_H (200 MHz; ^2H_2O) 2.22 (3 H, s, $COCH_3$), 3.18 (2 H, m, β-CH_2), 4.78 (2 H, q, OCH_2) and 3.95 (1 H, α-CH); δ_C (74.46 MHz; ^2H_2O) 29.41 (CH_3), 42.87 (β-CH_2), 50.25 (α-CH), 173.90 (CO_2H) and 211.23 (*C*OCH_3);

α-Trichloroethyl-*N*-trifluroacetyl-(2*S*)-aspartate (93)

To a suspention of (2*S*)-aspartic acid (100 mg, 0.75 mmol) in dry THF (15 cm^3) at 0 °C under an atmosphere of nitrogen was added dropwise trifluroacetic anhydride (0.42 cm^3, 3.0 mmol). The reaction mixture was allowed to stir at 0 °C for 1 h and

then at room temperature for a further 2 h, during which time dissolution was completed. The solvent was removed under reduced pressure to give the anhydride as a white solid. The anhydride was treated with dry 2,2,2-trichloroethanol (15 cm^3) and the solution stirred at 50 °C for 16 h under nitrogen. The solvent was removed under reduced pressure to give the crude ester. Recrystallization from ether/pet. ether gave a white solid (50 mg, 50%), m.p. 123-25 °C; *m/z* (Found: $[M + H]^+$, 359.9425. $C_8H_8NO_5F_3Cl_3$requires 359.9420); δ_H (200 MHz; C^2HCl_3) 2.99-3.35 (H, m, β-CH_2), 4.82 (2 H, q, OCH_2), 5.05 (1 H, pen., α-CH), 7.46 (1 H, d, NH) and 8.18 (1 H, br, CO_2H); δ_C (74.76 MHz; d_6-DMSO) 34.50 (β-CH_2), 49.79 (α-CH), 74.45 (OCH_2), 95.31 (CCl_3), 116.38 (CF_3, q, *J* 286.33), 156.84 (*C*ONH, q, *J* 24.65), 171.51 ($CO_2CH_2CCl_3$) and 171.91 (CO_2H); *m/z* (EI) 360, 362, 364 & 366 (3:3:2:1) (1%, M^+), 212 (17, $[M - OCH_2CCl_3]^+$) and 184 (100, $[M - CO_2CH_2CCl_3]^+$).

α-Trichloroethyl-β-methyl-*N*-trifluoroacetyl aspartic acid (94)

This was prepared in an identical manner to α-allyl β-methyl-*N*-trifluoroacetyl aspartic acid (96) using α-trichloroethyl-*N*-trifluroacetyl aspartic acid (**93**) in 80% yield; δ_H (200 MHz; C^2HCl_3) 2.89-3.29 (2 H, dd, β-CH_2), 3.71 (3 H, s, β-OCH_3), 4.78 (2 H, q, OCH_2), 5.01 (1 H, α-CH) and 7.60 (1H, d, NH); δ_C (50.3 MHz; C^2HCl_3) 33.45 (β-CH_2), 49.10 (α-CH), 52.99 (OCH_3), 74.54 (OCH_2), 107.88 (CCl_3), 168.09 (CO_2CH_3) and 171.54 (CO_2CCl_3).

α-Allyl-*N*-trifluroacetyl-(2*S*)-aspartate (95)

To a suspention of (2*S*)-aspartic acid (2 g, 15 mmol) in dry THF (40 cm^3) at 0 °C under an atmosphere of nitrogen was added dropwise trifluroacetic anhydride (8.46 cm^3, 60 mmol) over 30 min. The reation mixture was allowed to stir at 0 °C for

1 h and then at room temperature for a further 2 h, during which time dissolution was completed. The solvent was removed under reduced pressure to give the anhydride as a white solid. The anhydride was treated with dry allyl alcohol (45 cm^3) and the solution stirred at room temperature for 16 h under nitrogen. The solvent was removed under reduced pressure to give the crude allyl ester. Purification by column chromatography using ethyl acetate/pet. ether (1:1) as the eluant gave a white solid (3.8 g, 94%), m.p. 86-88 °C; $[\alpha]_D$ +27.4 (*c* 1, CH_2Cl_2); *m/z* (Found: $[M + H]^+$, 270.0597. $C_9H_{11}NO_5F_3$ requires 270.0589); υ_{max} (Nujol)/cm^{-1} 3296 (NH str), 1751 (ester C=O), and 1717 (trifluoroacetyl C=O); δ_H (200 MHz; C^2HCl_3) 2.95-3.27 (2 H, ABX, β-CH_2), 4.62-4.72 (2 H, m, OCH_2), 4.80-4.95 (1 H, m, α-CH), 5.27-5.39 (2 H, m, $CH{=}CH_2$), 5.80-5.99 (1 H, m, $CH{=}CH_2$), 7.39-7.43 (1 H, d, NH) and 9.43 (1 H, br, CO_2H); δ_C (74.76 MHz; C^2HCl_3) 35.16 (β-CH_2), 48.73 (α-CH), 67.23 (OCH_2), 115 (CF_3), 119 ($CH{=}CH_2$), 130.85 ($CH{=}CH_2$), 157.50 (CONH), 168.82 (CO_2CH_2) and 175.68 (CO_2H); *m/z* (CI) 270 (100%, $[M + H]^+$).

α-Allyl β-methyl *N*–trifluoroacetyl aspartic acid (96)

To a stirred solution of the α-allyl *N*-trifluroacetyl aspartic acid **(95)** (150 mg, 0.53 mmol) in ether was added slowly, dropwise ethereal diazomethane at 0 °C. After the addition was complete, the ice bath was removed and the reaction mixture was stirred at room temperature for 2 h. Excess diazomethane was flushed with N_2 and the solvent was removed under reduced pressure to give the crude α-allyl β-methyl aspartic acid ester. Purification by silica chromatography using ethyl acetate/ pet. ether (1:1) as the eluant gave white crystals (130 mg, 83%), m.p. 57-61 °C; δ_H (300 MHz; C^2HCl_3) 2.84-3.22 (2 H, dd, β-CH_2), 3.70 (3 H, s, β-OCH_3), 4.58-4.69 (2 H, m, OCH_2-CH), 4.85-4.87 (1 H, m, α-CH), 5.24-5.38 (2 H, m, $CH{=}CH_2$), 5.81-5.93 (1 H, m, $CH{=}CH_2$) and 7.52 (1 H, d, NH); δ_C (50.3 MHz; C^2HCl_3) 35.67 (β-CH_2), 49.35 (α-CH), 52.82 (OCH_3), 67.41 (OCH_2-CH), 119.78 ($CH{=}CH_2$), 131.39

($CH{=}CH_2$), 157.68 (CONH), 169.21 (CO_2CH_2) and 171.54 (CO_2CH_3); *m/z* (EI) 283 (1%, M^+), 252 (3, $[M - OCH_3]^+$), 242 (2, $[M - C_3H_5]^+$), 238 (4, $[M - CH_2OCH_3]^+$), 224 (23, $[M - CO_2CH_3]^+$), 198 (100, $[M - CO_2C_3H_5]^+$) and 41 (92, $C_3H_5^+$).

α-Allyl-*N*-trifluoroacetyl-(2*S*)-aspartyl-β-acid chloride (97)

To a stirred solution of α-allyl-*N*-trifluroacetyl-(2*S*)-aspartic acid (**95**) (3.8 g, 14.11 mmol) in dry dichloromethane (20 cm^3) was added distilled thionyl chloride (3.08 cm^3, 42.33 mmol) and the mixture was reflux for 12 h under nitrogen. The solvent was removed under reduced pressure to yield the crude acid chloride (3.8 g, 94%), m.p. 89-90 °C; υ_{max} (Nujol)/cm^{-1} 3325 (NH str), 1819 (acid chloride C=O), 1741 (ester C=O) and 1712 (trifluoroacetyl C=O); δ_H (300 MHz; C^2HCl_3) 3.53-3.69 (2 H, m, β-CH_2), 4.56-4.70 (2 H, m, OCH_2), 4.77-4.82 (1 H, m, α-CH), 5.26-5.37 (2 H, m, $CH{=}CH_2$),5.81-5.94 (1H, m, $CH{=}CH_2$) and 7.38 (1 H, d, NH); δ_C (74.76 MHz; C^2HCl_3), 47.40 (β-CH_2), 49.33 (α-CH), 67.65 (OCH_2), 115.48 (CF_3, q, *J* 286.5), 120 ($CH{=}CH_2$) 130.56 ($CH{=}CH_2$), 157.33 (CONH, *J* 37), 167.79 (CO_2OCH_2) and 172.25 (COCl); *m/z* (EI) 288 and 290 (4 and 1.3%, chlorine isotopes, $[M + H]^+$), 252 (26, $[M - Cl]^+$), 224 (86, $[M - COCl]^+$), 202 (37, $[M - CO_2C_3H_5]^+$) and 41 (100, $C_3H_5^+$).

α-Allyl 5-diazo-4-oxo-*N*-trifluoroacetyl-(2*S*)-norvalinate (98)

To a stirred solution of the α-allyl *N*-trifluoroacetyl-(2*S*)-aspartyl-β-chloride (**97**) (3.7 g, 12.86 mmol) in diethyl ether (40 cm^3) and tetrahydrofuran (10 cm^3) was added dropwise an excess of freshly prepared ethereal diazomethane (170 cm^3) over 30 min at 0 °C. The solution was allowed to warm to room temperature during 90 min after which time excess diazomethane was removed using a stream of nitrogen. The solvent

was then removed under reduced pressure to give a pale yellow solid which was recrystallised from diethyl ether/ pet.ether to give yellow crystals of the diazoketone (3.5 g, 93%), m.p. 53-54 °C; δ_H (300 MHz; C^2HCl_3) 2.87-3.27 (2 H, ABX, β-CH_2), 4.61-4.68 (2 H, m, OCH_2), 4.80-4.82 (1 H, m, α-CH), 5.25-5.35 (2 H, m, CH=CH_2), 5.82-5.95 (1 H, m, CH=CH_2) and 7.64 (1 H, d, NH); δ_C (74.76 MHz; C^2HCl_3) 40.27 (β-CH_2), 49.09 (α-CH), 55.81 (CHN_2),66.91 (OCH_2), 115.72 (CF_3, q, J 286.5), 119.28 (CH=CH_2), 131.20 (CH=CH_2), 156.15 (CF_3CONH, q, J 37.5), 169.05 (CO_2CH_2) and 191.46 ($COCHN_2$).

α-Allyl 4-Oxo-*N*-trifluroacetyl-(2*S*)-norvalinate (99)

Aqueous HI (55%; excess) was added dropwise with stirring to a solution of the diazoketone (**70**) (2.50 g, 8.52 mmol) in dichloromethane (40 cm^3). After 15 min the organic phase was washed with water (2 x 10 cm^3), dilute aqueous sodium thiosulfate (until colourless), water (5 cm^3) and then dried ($NaSO_4$). The solvent was removed under reduced pressure to yield a yellow solid which was recrystallised from diethyl ether/ pet. ether to give white crystals (1.90 g, 84%), m.p. 53-54 °C; $[\alpha]_D$ +39.3 (*c* 1, CH_2Cl_2); *m/z* (Found: $[M - C_3H_5]^+$, 226.0336. $C_7H_7NO_4F_3$ requires 226.0327); υ_{max} (Nujol)/cm^{-1} 3336 (NH str), 1748 (ester C=O) and 1722 (trifluoroacetyl C=O and ketone C=O); δ_H (300 MHz; C^2HCl_3) 2.18 (3 H, s, CH_3), 2.98-3.35 (2 H, ABX, β-CH_2), 4.58-4.66 (2 H, m, OCH_2), 4.68-4.78 (1 H, m, α-CH), 5.23-5.34 (2 H, m, CH=CH_2), 5.79-5.92 (1 H, m, CH=CH_2) and 7.43-7.45 (1 H, d, NH); δ_C (74.76 MHz; C^2HCl_3) 29.77 ($COCH_3$), 43.90 (β-CH_2), 48.86 (α-CH), 66.85 (OCH_2), 115.7 (CF_3, q, J 285.75), 119.35 (CH=CH_2), 131.15 (CH=CH_2), 157.132 (CONH, q, J 37.5), 169.15 (CO_2CH_2) and 206.55 ($COCH_3$); *m/z* (EI) 267 (1%, M^+), 226 (12, $[M - C_3H_5]^+$), 43 (100, $[M - COCH_3]^+$) and 41 (45, $C_3H_5^+$).

Chapter Four

REFERENCES

4.0 REFERENCES

1. E. F. Snell, *J. Am. Chem. Soc.*, 1945, **67**, 194.
2. Enzyme Nomenclature. Recommendations (1992) of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology. Academic Press, 1992.
3. D. R. Curtis and G. A. R. Johnston, *Ergebn. Physiol.*, 1974, **69**, 98-167.
4. O. Heby, *Adv. Enz. Regul.*, 1985, **24**, 103-124.
5. C. G. Kannangara, R. V. Andersen, B. Pontoppidan, R. Willows and D. Von Wettstein, in *Ciba Foundation Symposium 180. The biosynthesis of the tetrapyrrole Pigments*, John Wiley & Sons, New York, 1994, p 3-25.
6. D. Dolphin, R. Poulson and O. Avramovic (eds.), *Vitamin B_6; Pyridoxal Phosphate, Chemical, Biochemical and Medical Aspects,* Wiley, New York, 1986.
4. A. E. Evangelopolous (ed.), *Chemical and Biological Aspects of Vitamin B_6 Catalysis, Part A and B*, Liss, New York, 1984.

7. C. Tabor and H. Tabor, *Ann. Rev. Biochem.*, 1976, **45**, 285.
8. D. H. Russel, *Polyamines in Natural and Neoplastic Growth*, Raven, New York, 1973.
9. International Encyclopedia of pharmacology and Therapeutics, Section 74, *Histamines and Antihistamines*, ed. M. Schachter., Pergamon press, Oxford, 1973.
10. C. Walsh, *Enzymic Reaction Machanisms*, W. H. Freeman and Company, U.S.A., 1979, p 80.
11. M. J. Jung, *Bioorg. Chem.*, 1986, **14**, 424.
12. J. T. Park, *Symp. Soc. Gen. Microbiol.*, 1958, **8**, 49.
13. C. Walsh, R. Badet, E. Damb, N. Esaki and N. Golakatos, *Spec. Publ. Roy. Soc. Chem.*, 1986, **55**, 193.

14. F. C. Neuhans, in *Antibiotics*, ed. D. Gottlieb and P. D. Shaw, Springer-Verlag, New York, 1967, vol 1, p. 40.

15. A. E. Braunstein and M. M. Shemyakin, *Biokhymiya*, 1953, **18**, 393-411.

16. D. E. Metzler, M. Ikawa and E. E. Snell, *J. Am. Chem Soc.*, 1954, **76**, 648-652.

17. J. K. Kirsch, G. Eichele, G. C. Ford, M. G. Vincent, J. N. Jansonius, H. Gehring and P. Christen, *J. Mol. Biol.*, 1984, **174**, 497.

18. P. Besmer and D. Arigoni, *Chimia*, 1969, **23**, 190.

19. H. C. Dunathan, L. Davis, P. Kury and M. Kaplan, *Biochem.*, 1968, **7**, 4532.

20. H. C. Dunathan, *Proc., Natl., Acad., Sci. USA*, 1966, **55**, 712.

21. P. Christen and D.E. Metzler (eds.), *Transaminases*, Wiley, New York, 1985.

22. A. Irite, H. A. Farach and M. Martinez-Carrion, *J. Biol. Chem.*, 1984, **259**, 7003.

23. A. M. Garzillo, G. Marino and B. Pispisa, *FEBS Lett.*, 1984, **170**, 223.

24. C. A. Vermon, *Biochem. Soc. Trans.*, 1984., **12**, 420.

25. G. C. Ford, G. Eichele and J. N. Jansonius, *Proc., Natl., Acad., Sci. U.S.A.*, 1980, **77**, 2559.

26. A. Arnone, P. D. Briley, P. H. Royer, C. C. Hyde, C. M. Metzler and D. E. Metzler in *Molecular structure and Biological Activity*, eds. J. F. Griffen and W. L. Duowe, Elsevier, North Holland Inc., New York, 1982, p. 57-74.

27. A. E. Barunstein, E. G. Harutyunyan, V. N. Malashkevich, V. M. Kochikina and Y. M. Torchinsky, *Mol. Biol. (Moscow)*, 1985, **19**, 196.

28. S. Kamitori, A. Okamoto, K. Hirotsu, T. Higuchi, S. Kuramitsu, H. Kagamiyama, Y. Matsuura and Y. Katsube, *J. Biochem. (Tokyo)*, 1990, **108**, 175.

29 H. C. Dunathan, *Adv. Enzmol.*, 1971, **35**, 79.

30. Y.M. Torchinsky, *Aspartate Aminotrasferase* in *Coenzymes and Cofactors; Vitamin B_6, Pyridoxal Phosphate,* eds. D. Dolphin, R. Poulson and O. Avramovic, John Wiley & Sons, New York, 1986, vol. 1, Part B, p. 169.

31. D. M. Kiick and P. F. Cook, *Biochemistry*, 1983, **22**, 375-382.

32. G. C. Ford, G. Eichele and J. N. Jansonius, *Proc., Natl., Acad., Sci. USA*, 1980, **77**, 2559.

33. C. Walsh, *Enzymatic Reaction Mechanisms*, W. H. Freeman and Company, U.S.A., 1979, p. 782.

34. P. Christen and D. E. Metzler, *Transaminases*, Wiley, New York, 1985, vol. 2, p. 178.

35. S. Doonan, D. Barra and F. Bossa, *Int. J. Biochem.*, 1984, **16**, 1193.

36. A. E. Braunstein, in *The Enzymes*, ed. P. D. Boyer, Academic Press, 1973, vol. **9**, chap. 10.

37. B. Boettcher and M. Martinez-Carrion, *Biochemistry*, 1976, **15**, 5657.

38. H. Schlegel, P. E. Zaorlek and P. Christen, *J. Biol. Chem.*, 1977, **252**, 5835.

39. D. Gani, in *Comprehensive Medicinal Chemistry*, ed. P. G. Sammes, Pergamon Press, Oxford, 1990, vol **2**, p. 213-245.

40. D. M. Smith, N. R. Thomas and D. Gani, *Experientia*, 1991, **47**, 1104.

41. B. Grimm, *Proc. Natl. Acad. Sci. U.S.A.*, 1990, **87**, 4169.

42. B. Grimm, A. J. Smith, C. G. Kannangara and M. Smith, *J. Biol. Chem.*, 1991, **266**, 12495.

43. M. A. Smith, B. Grimm, C. G. Kannangara and D. VonWettstein, *Eur. J. Biochem.*, 1991, **202**, 749.

44. B. Grimm, A. Bull, K. G. Welinder, S. P. Gough and C. G. Kannangara, *Carlsberg Res. Commun.*, 1989, **54**, 67.

45. W. Y. Wang, D. D. Huang, D. Stachon, S. P. Gough and C. G. Kannangara, *Plant Physiol.*, 1984, **74**, 569.

46. M. A. Smith, C.G Kannangara and B. Grimm, *Biochemistry*, 1992, **31**, 11249.

47. C. E. Pugh, J. L. Harwood, and R. A. John, *J. Bio. Chem.*, 1992, **267**, 1584.

48. S. Brody, J. S. Andersen, C. G. Kannangara, M. Meldgaard, P. Roepstorf and D. Von Wettstein, *Biochemistry*, 1995, **34**, 15918.

49. L. L. Henderson and R. B. Johnston, *Biochem. Bophys. Res. Commun.*, 1976, **68**, 793.

50. H. Yamada and M. H. O' Leary, *Biochemistry*, 1978, **17**, 669.

51. D. Voss, J. Gerdee and E. Leistner, *Phytochemistry*, 1985, **24**, 1471.

52. E. Santaniello, M. G. Kienle, A. Manzocchi and E. Bosisio, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1677.

53. A. R. Battersby and R. J. Staunton, *FEBS Lett.*, 1975, **55**, 265.

54. A. R. Battersby, M. Nicoletti, J. Staunton and R. Vleggaar, *J. Chem. Soc., Perkin Trans. 1*, 1980, 43.

55. A. R. Battersby, E. J. T. Chrystal and J. Staunton , *J. Chem. Soc., Perkin Trans. 1*, 1980, 31.

56. A. R. Battersby, R. Murphy and J. Staunton , *J. Chem. Soc., Perkin Trans. 1*, 1982, 449.

57. G. R. Orr and S. J. Gould, *Tetrahedron Lett.*, 1982, **23**, 3139.

58. J. C. Richards and I. D. Spenser, *Can. J. Chem.*, 1982, **60**, 2810.

59. D. E. Stevension, M. Akhtar and D. Gani, *Tetrahedron Lett.*, 1986, **27**, 5661.

60. J. W. Thanassi and J. S. Fruton, *Biochemistry*, 1961, **1**, 975.

61. J. G. Kelland, M. M. Palcic, M. A. Pickard and J. W. Vederas, *Biochemistry*, 1985, **24**, 3263.

62. Y. Asada, K. Tanizawa, S. Sawada, T. Susuki, H. Misono and K. Soda, *Biochemistry*, 1981, **20**, 6681

63. L. Schirch and T. gross, *J. Biol. Chem.*, 1968, **243**, 5651.

64. M. Akhtar, H. A. El-Obeid and P. M. Jordan, *Biochem. J.*, 1975, **145**, 159.

65. R. J. Ulevitch and R. G. Kallen, *Biochemistry*, 1977, **16**, 5342.

66. R. J. Ulevitch and R. G. Kallen, *Biochemistry*, 1977, **16**, 5350.

67. R. J. Ulevitch and R. G. Kallen, *Biochemistry*, 1977, **16**, 5355.

68. A. G. Palekar, S. S. Tate, and A. Meister, *J. Biol. Chem.*, 1973, **248**, 1158.

69. J. G. Voet, D. M. Hindenlang, T. J. J. Blanck, R. J. Ulevitch, R. G. Kallen and H. C. Dunathan, *J. Biol. Chem.*, 1973, **248**, 841.

70. L. Schirch and W. T. Jenkins, *J. Biol., Chem.*, 1964, **239**, 3797.

71. L. Schirch and W. T. Jenkins, *J. Biol., Chem.*, 1964, **239**, 3801.

72. J. Hansen and L. Davis, *Biochem. Biophys. Acta*, 1979, **568**, 321.

73. N. R. Thomas, J. E. Rose and D. Gani, *T. C. S. Chem. Comn.*, 1991, 908

74. L. Davis, D. E. Metzler, in *The Enzymes*, ; ed. P. D. Boyer, Academic press, New York, 1972, 3rd ed. vol. 7, p. 33-74.

75. J. E. Churchich, in ref. 2, p. 311.

76. R. Tamada, Y. Wakabayashi, A. Iwashima and T. Hasegawa, *Biochim. Biophys. Acta*, 1986, **871**, 279.

77. G. G. Hammes and J. L. Haslam, *Biochemistry*, 1986, **7**, 1519.

78. C. T, Walsh, in ref. 2, p. 43.

79. M. Johnston and C. T. Walsh, in *Molecular Basis for Drug Action*, eds. T. Singer and Ondarza, Elsevier, Amsterdam, 1981, p. 167.

80 . C. Danzin and M. J. Jung, in ref. 4, p. 377.

81. R. Rando, *Biochemistry*, 1974, **13**, 3859.

82. H. Gehring, R. Rando and P. Christen, *Biochem.*, 1977, **16**, 4832.

83. A. Cooper, S. Fitzpatrick, C. Kaufman and P. Dowd, *J. Am. Chem. Soc.*, 1982, **104**, 332.

84. R. Rando, N. Relyea and L. Cheng, *J. Biol. Chem.*, 1976, **251**, 3306.

85. J. P. Scannnell, D. L. Preuss, T. C. Demmy, F. Weiss, T. Williams and A. Stempel, *J. Antibiot.*, 1971, **4**, 239.

86. Y. Morino and M. Okamoto, *Biochem. Biophys.Res. Commun.*, 1973, **50**, 1061.

87. Y. Morino, A. M. Osman and M. Okamoto, *J. Biol. Chem.*, 1974, **249**, 6684.

88. R. A. John and P. Fasella, *Biochemistry*, 1969, **8**, 4477.

89. H. Ueno, J. J. Likos and D. E. Metzler, *Biochemistry*, 1982, **21**, 4387.

90. T. Alston and H. Bright, *FEBS Lett.*, 1981, **126**, 269.

91. R. M. Khomutov, E. S. Severin, G. K. Kovaleva, N. N. Gulyaev, N. U. Gnuchev and L. P. Sastchenko, in *Pyridoxal Catalysis: Enzymes and Model Systems*, eds. E. E. Snell, A. E. Braunastein, E. S. Severin and Y. M. Torchinsky, Wiley, 1968, p. 631.

92. G. K. Kovaleva, E. S. Severin, P. Fasella and R. M. Khomutov, *Biokhimiya,* 1973, **38**, 365.

93. G. K. Kovaleva and E. S. Severin, *Biokhimiya*, 1972, **37**, 1282.

94. A. E. Braunstein, in *The Enzymes*, ed. P. D. Boyer, Academic Press, 1973, 3 rd Edn., vol. **9**, p. 443.

95. L. J. Fowler and R. A. John, *Biochem. J.*, 1972, **130**, 569-573.

96. R. Rando and F. W. Bangerter, *J. Am. Chem. Soc.*, 1976, **98**, 6762-6764.

97. R. Rando, *Biochemistry*, 1977, **16**, 4604-4610.

98. E. Wang and C. Walsh, *Biochemistry*, 1978, **17**, 1313-1321.

99. D. Roise, N. Esaki, K. Soda and C. Walsh, *Biochemistry*, 1984, **23**, 5195-5201.

100. B. Badet, D. Roise and C. Walsh, *Biochemistry*, 1984, **23**, 5188-5194.

101. K. Inagaki, K. Tanizawa, B. Badet, C. T. Walsh, H. Tanaka and K. Soda, *Biochemistry*, 1986, **25**, 3268-3274.

102. B. Badet, K. Inagaki, K. Soda and C. T. Walsh, *Biochemistry*, 1986, **25**, 3275-3287.

103. M. D. Toney, E. Hohenester, S, W. Cowan and J. N. Jansonius, *Science*, 1993, **261**, 756-759.

104. A. Maycock, S. Aster, A. Patchett, in *Enzyme-Activated Irreversible Inhibotors,* Elsvier, New York, 1987, p. 211.

105. G. Gayon-Ribereau, C. Danzin, M. Palfreyman, M. Aubry, J. Wagner, B. Metcalfe and M. Jung, *Biochem. Pharmacol.*, 1979, **28**, 1331.

106. M. Palfreyman, C. Danzin, P. Bey, M. Jung, G. Gayon-Ribereau, M. Aubry, J. Vevert and A. Sjoerdsman, *J. Neurochem.*, 1978, **31**, 927.

107. B. Metcalfe, P. Bey, C. Danzin, M. Jung, P. Casara and J. Vevert, *J. Am. Chem. Soc.*, 1978, **100**, 2551.

108. R. Rando and N. Relyea, *Biochem. Biophys. Res. Commun.*, 1975, **67**, 392.

109. C. Danzin, P. Bey, D. Schirlin and N. Claverie, *Biochem. Pharmacol.*, 1982, **31**, 3871

110. P. Bey, F. Gerhart, V. V. Dorsselaer and C. Danzin, *J. Med. Chem.*, 1983, **26**, 1551.

111. A. Phillips, *Biochim. Biophys. Acta*, 1968, **151**, 523-526.

112. H. Yoshida, K. Hanada, H. Oshawa, H. Kamagai and H. Yamada, *Agric. Biol. Chem.*, 1982, **46**, 1035-1042.

113. K. D. Schnackerz, J. H. Ehrlich, W. Geisemann and T. A. Reed, *Biochemistry*, 1979, **18**, 3557-3563.

114. E. Groman, Y. Z. Huang, T. Watanabe and E. E. Snell, *Proc. Natl. Acad. Sci. USA*, 1972, **69**, 3297-3300.

115. I. Giovanelli, L. Owens and S. H. Mudd, *Biochim. Biophys. Acta*, 1971, **227**, 671-684.

116. R. B. Silverman and R. Abeles, *Biochemistry*, 1976, **15**, 4718-4723.

117. E. W. Miles, *Biochem. Biophys. Res. Commun.*, 1975, **64**, 248-252.

118. J. Kovacs, H. Nagy Kovacs and R. Ballina, *J. Am. Chem. Soc.*, 1963, **85**, 1839.

119. J. Kovacs, H. N. Kovacs, I. Konyves, J. Csazar, T. Vajda and H. Mix, *J. Org. Chem.*, 1961, **26**, 1084.

120. C. Ressler, *J. Am. Chem. Soc.,* 1960, **82**, 1641.

121. D. Gani and D. W. Young, *J. Chem. Soc., Perkin Trans. I,* 1983, 2393.

122. J. E. Baldwin, M.G. Malony and M. North, *Tetrahedron*, 1989, **45**, 6331.

123. M. Goodman and F. Boardman, *J. Am. Chem. Soc.*, 1963, **85**, 2483.

124. H. Schwarz, F. M. Bumpus and I. H. Page, *J. Am. Chem. Soc.*, 1957, **79**, 5697.

125. P. Gmeiner, P. L. Feldman, M. Y. Chu-Moyer and H. Rapoport, *J. Org. Chem.*, 1990, **55**, 3068.

126 J. Jansonius and M. G. Vincent, in *Biological Macromolecules and Assemblies*, eds. F. A. Jurnak and A. McPherson, John Wiley & Sons, New York 1987, vol. 3, pp. 187-285.

127. M. S. Okamoto and E. K. Barefield, *Inorganic chemistry*, 1974, **13** , 2611.

128. M. Noji, S. Motoyama, T. Tashiro and Y. Kidani, *Chem. Pharm. Bull.*, 1983, **31**, 1469.

129. M. Waki, Y. Kitajima and N. Izumiya, *Synthesis,* 1981, 266.

130. A. Previero, J. C. Cavadorw, J. Torreilles and M. A. Coletti-Previero, *Biochimica Biophysica Acta*, 1979, **581**, 276.

131. L. Davis and D. Metzler in *'The Enzymes'*, ed. P. D. Boyer, Academic Press, New York, 1972, 3rd edn., vol.7, p. 33.

132 (a) G. E. Syke, R. Potts and H. G. Floss, *J. Am. Chem. Soc.*, 1974, **96**, 1593;
(b) H. G. Floss and E. Schieicher, *J. Biol. Chem.*, 1976, **251**, 5478;
(c) Y. F. Cheung and C. W. Howards, *J. Am. Chem. Soc.*, 1976, **98**, 3397.

133. C. Fuganti, D. Ghiringhelli, D. Giangrasso, P. Grasselli and A. S. Amisano, *Chim. Ind.* (Milan), 1974, **56**, 424.

134. F. Weygand, P. Klinke and I. Eigen, *Chem. Ber.*, 1957, **90**, 1896.

135 R.B. Woodward, K. Hensler, J. Gosteli, R. Naegeli, W. Oppolozer, R. Ramage, S. Ranganathan and H. Varbrugen, *J. Am. Chem. Soc.*, 1966, **88**, 852.

136. W. C. Still, M. Khan and A. Mitra, *J. Org. Chem.*, 1978, **43**, 2923.

137. J. P .Greenstein and M.Winitz, in *Chemistry of the Amino acids*, Kreger, vol 2.

138. J. Rose, *Ph.D. Thesis*, St. Andrews, 1992.